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(71) Applicant: HORUS THERAPEUTICS, INC. [US/US]; 2320 Brighton-Henrietta Town Line Road, Rochester, NY 14623 (US).

(72) Inventors: BARNHILL, Stephen, D.; 19 Mad Turkey Crossing, Savannah, GA 31411 (US). ZHANG, Zhen; 2055 Middleburg Lane, Mt. Pleasant, SC 29464 (US).

(74) Agents: TAUTVYDAS, Daiva, K. et al.; Jones & Askew, 37th floor, 191 Peachtree Street, N.E., Atlanta, GA 30303-1769 (US).

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(54) Title: COMPUTER ASSISTED METHODS FOR DIAGNOSING DISEASES

(57) Abstract

The present invention is directed to an *in vitro*, biological fluid-based method for diagnosing, screening or prognosing for diseases in which laboratory data are manipulated to obtain a diagnostic index. In one embodiment, a neural network is used to obtain the diagnostic index.

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COMPUTER ASSISTED METHODS FOR DIAGNOSING DISEASES

Cross Reference to Related Applications

The present application is a continuation-in-part of co-pending U.S. Application Serial Number 08/315,851 filed on September 30, 1994, which is a continuation of U.S. Application Serial Number 07/990,772 filed on December 14, 1992, now abandoned, which is a continuation-in-part of U.S. Application Serial Number 07/964,486 filed on October 21, 1992, now abandoned, which is a continuation of U.S. Application Serial Number 07/806,980 filed on December 12, 1991, now abandoned.

Technical Field

The present invention relates to methods for diagnosing, screening or prognosing diseases. More particularly, the present invention relates to a method for diagnosing, screening or prognosing diseases in humans or animals, and for determining the severity and cause of the disease, by measuring blood serum levels of specific, predetermined blood constituents and then calculating a diagnostic index based on the relationships between those blood constituents. Optionally, demographic information, such as the age, race or sex of the patient, as well as the patient's medical history can be factored into the diagnosis.

The present invention further relates to an in vitro, serum-based computer assisted method for diagnosing, screening or prognosing diseases, utilizing a neural network to obtain a diagnostic index. In preferred embodiments of the present invention, the method is used to diagnose osteopenia and certain cancers, including but not limited to ovarian, breast, testicular, colon and prostate cancer.

Background of the Invention

As used herein, the term "disease" is defined as a deviation from the normal structure or function of any part, organ or system of the body (or any combination thereof). A specific disease is manifested by characteristic symptoms and signs, including both chemical and physical changes. Certain characteristic signs and symptoms can be quantitated by clinical chemical analysis to yield important diagnostic information. For purposes of this application, the quantifiable signs, symptoms and/or analytes in biological fluids characteristic of a particular disease are defined as "biomarkers" for the disease. Current diagnostic methods depend on the identification and evaluation of these biomarkers, both individually and as they relate to one another.

Generally, the pathological process involves gradual changes that become apparent only when overt change has occurred. In many instances, pathological changes involve subtle alterations in multiple biomarkers. It is uncommon that a single biomarker will be indicative of the presence or absence of a disease. It is the pattern of those biomarkers relative to one another and relative to a normal reference range, that is indicative of the presence of a disease.

When individual biomarkers do not show a predictable change and the patterns and interrelationships among the biomarkers viewed collectively are not clear, the accuracy of a physician's diagnosis is significantly reduced. Also, as the number of biomarkers significant for a particular disease increases, the number of diagnostic patterns increases, correspondingly, pattern recognition and accuracy of a physician's diagnosis decreases.

For example, the following biomarkers collectively show characteristic changes in the presence of osteoporosis: calcium, phosphate, estradiol (follicular, mid-cycle, luteal, or post-menopausal), progesterone (follicular, mid-cycle, luteal, mid-luteal, oral contraceptive, or over 60 years), alkaline phosphatase, percent liver-ALP, and total intestinal-ALP. After measuring these biomarkers, a diagnosing physician would next compare the measuring to a normal reference range. While some of the biomarkers may fall outside the normal reference range, others may fall clearly within the normal reference range. In some circumstances, all of the biomarker values may fall within a normal reference range. Presented with

such data, a physician may suspect that a patient has undergone some bone loss, but will be unable to reach a conclusive and meaningful diagnosis as to the presence of the disease osteoporosis.

The characteristic changes in biomarkers associated with some diseases are well documented; however, the quantitative interpretation of each particular biomarker in diagnosing a disease and determining a prognosis is not well established. The difficulties inherent in formulating a diagnosis from the analysis of a set of laboratory data is best illustrated by looking closer at conventional diagnostic methods for a specific disease. A discussion of the disease osteoporosis follows.

The term "osteopenia" as used herein means any decrease in bone mass below the normal. The term "osteoporosis" as used herein means a specific form of generalized osteopenia characterized by a decrease in bone density, low bone mass, and microarchitectural deterioration of bone tissue.

Osteopenia encompasses a group of diseases with diverse etiologies typified by reduction in bone mass per unit volume to a level below that which is necessary for adequate mechanical support. Osteoporosis is the result of the gradual depletion of the inorganic portion of the skeleton and can be caused by any number of factors. Primary osteoporosis is an age related disorder that is particularly common in women and is characterized by decreased bone mass in the absence of other recognizable causes. However, osteoporosis occurs in both men and women. In women it is recognized usually at the 5th or 6th decade, following menopause. In men osteoporosis is often recognized around their 6th or 7th decade.

The following is a partial list of some of the categories of individuals at risk for developing osteoporosis:

Post-menopausal women
Cigarette smokers
Heavy users of alcohol
Users of a variety of drugs, such as steroids
Female runners and ballet dancers
Male marathoners consuming two few calories
Bulimics and anorexics
People with poor diets
People allergic to dairy products

People affected with cancer
Fair and slim women
All men and women over the age of 65.

In addition to being female, the three most significant risk factors are poor diet, lack of exercise, and being postmenopausal. Other risk factors which are associated with osteoporosis include Caucasian or Oriental ancestry, a fair complexions, and a family history of osteoporosis.

The onset of osteoporosis may be insidious or sudden, following trauma. The most common complaint associated with osteoporosis is back pain. Eventually, the pain may spread to the pelvis, the thorax, and the shoulders. In the spine, the vertebrae can compress, and the back can take on a "bent" appearance. Conditions such as kyphosis (humpback) or scoliosis may occur. If the spine becomes deformed, other body parts can be affected as well. For example, the ribs can be pushed against the pelvis, or the stomach can be pushed into the pelvis. In addition to spinal problems, osteoporosis can also lead to fractures of the hip, wrist, and ribs. These fractures can occur with only slight trauma and sometimes with no trauma at all. *Mazess B., et al.*, "Bone Density of the Radius, Spine, and Proximal Femur in Osteoporosis," *J. of Bone and Mineral Research*, Vol 3, pgs. 13-18, (1988); *Riggs B. L., et al.*, "Involutional Osteoporosis", *New Engl. J. Med.*, Vol. 314, pgs. 1676-1686, (1986). The changes associated with osteoporosis are gradual so osteoporosis is often not detected in its early stages.

From the research perspective, osteoporosis can be classified as either primary or secondary to another disease. Primary osteoporosis is further classified as juvenile, idiopathic, postmenopausal (Type I) and involutional (Type II). It is now understood that accelerated bone loss occurs with cessation of menstruation at the time of menopause and in women who have amenorrhea as a result of prolactin producing pituitary tumor, anorexia nervosa, or intense long-distance running associated with undernourishment. These situations are all accompanied by estrogen deficiency which is likely to be a major determinant of the accelerated bone loss. Bone loss also occurs when estrogen therapy is withdrawn.

Bone consists primarily of an extracellular matrix containing (by weight) approximately 35% organic and 65% inorganic components. The

cells of bone represent a minor component of the bone constituents, yet they carry out a major portion of the function of the skeletal system. Bone cells help maintain serum calcium concentration within a narrow range to regulate mineral homeostasis while also being responsible for the continuous formation and resorption of the extracellular matrix, allowing response of the skeletal system to the mechanical forces resulting from physical activity.

The major organic component of the extracellular matrix is collagen. This protein has a rigid rodlike structure and is composed of three alpha chains held together in helical fashion by covalent and noncovalent forces. Multiple collagen molecules form fibrils, and these fibrils in turn are arranged in bundles or fibers. It is the bundles or fibers of collagen that can be seen in the light microscope as layers or linear arrays.

Non-collagen components of bone compose a very small portion of the organic matrix of the skeleton. This minor fraction consists of proteins, glycoproteins, lipids and mucopolysaccharides. Only a few of these components have been carefully identified and characterized. Two of the proteins that have been isolated and studied are osteonectin and osteocalcin.

Calcium and phosphorus are the main components of the inorganic portion of the skeleton. Initially, calcium and phosphorus are deposited as amorphous salts but later undergo rearrangements into a crystalline structure that resembles hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). Several other ions, including Na, K, Mg, and CO_3 in varying proportions, may be found in the skeletal hydroxyapatite. If there has been fluoride intake, there will also be fluoride in the hydroxyapatite.

Although considerable effort has been expended in studying the mechanism of bone mineralization, it is not fully understood. Several theories have been suggested to explain the available data. One theory is that calcium and phosphorus ions are present in the extracellular fluid in amounts exceeding the solubility product of [Ca] x [P]. These ions are kept from precipitating by inhibitors of calcification such as pyrophosphate. Because osteoblasts contain large amounts of alkaline phosphatase, it has been speculated that the activity of this enzyme facilitates mineralization by cleaving the phosphate groups, thus altering the Ca:P ratio in the sites of calcification.

In the clinical syndrome of osteoporosis, the reduction in bone mass can be attributed to osteopenia because of a dietary deficiency or absorption interference of proteins or Vitamin C. It can also be the result of a deficient stress stimulus. Osteopenia can also be caused by osteomalacia, a failure of proper mineralization of osteoid resulting from bone calcium or phosphorus deficiency or both. It can also be caused by insufficient absorption from the intestine due to lack of calcium or a resistance to the action of Vitamin D due to failure of its conversion to the biologically active forms, 25 hydroxychole-calciferol and 1.25 dihydroxychole-calciferol, formed by the liver and kidney, respectively. In addition, it may well be caused by an abnormal rate of osteolysis due to parathyroid hormone stimulation of osteoclastic activity (osteocasts are hematopoitic in origin, arising from the migration of monocytes to bone). In reality, most cases of osteoporosis, when carefully analyzed, reveal evidence of the causes of osteopenia.

Chemical analysis of blood may reveal calcium, phosphorus, and alkaline phosphatase within the normal range. However, an isoenzyme of alkaline phosphatase may be significantly increased. Increased bone resorption seen in osteoporotic patients, which occurs as a result of the action of osteoclasts, usually involves the dissolution of both minerals and organic matrix eventually leading to increased excretion of urinary hydroxyproline. Serum estradiol which is secreted almost entirely by the ovary is significantly decreased in these patients. This observation is further corroborated where it has been demonstrated that exogenous estrogen therapy in perimenopausal women does delay the onset of post menopausal osteopenia. Most experts agree that estrogen appears to decrease bone resorption. *Weiss N. S., et al.*, "Decreased Risk of Fractures of the Hip and Lower Forearm with Postmenopausal Use of Estrogen," *N. Engl. J. Med.*, Vol. 303, pgs. 1195-1198 (1980); *Ettinger B., et al.*, "Long Term Estrogen Replacement Therapy Prevents Bone Loss and Fractures," *Ann. Intern. Med.*, Vol. 102, pgs. 319-324, (1985).

It is believed that blood levels of calcium are maintained without increasing calcium loss from the bone as long as there are normal amounts of estrogen and parathyroid hormone present. It is reasonably excepted that estrogen antagonizes the effect of parathyroid hormone. Estrogen deficiency, as seen in peri- and postmenopausal women, results in an increase in the sensitivity of bone to parathyroid hormone. This antagonistic

relationship eventually leads to an increase in the resorption of the bone and contributes to the development of Type I Osteoporosis. Type II Osteoporosis is characterized by reduced calcium absorption, which in turn results in increased secretion of parathyroid hormone. Type II Osteoporosis occurs in older individuals and is associated with wedge fractures of the spine and fractures of the hip. A theory recently advanced by *Barnhill, et al.* suggests that evidence of decreased serum estradiol and increased lymphocyte alkaline phosphatase, represents an activated immune system in osteopenic postmenopausal women. This finding suggests that "uncovered estrogen receptors" may induce an immune reaction which is responsible for one form of osteoporosis. *Barnhill S.D., et al.*, "Osteoporosis: A Possible Autoimmune Etiology," *Ann. of Clin. Lab. Sci.*, Vol. 17, pgs. 255-256, (1987).

In 1987, *Barnhill, et al.* proposed an autoimmune etiology for some forms of osteopenia. In that publication, *Barnhill, et al.* showed that lymphocyte-derived alkaline phosphatase was present in the blood of 90% of severely osteopenic women. See *Barnhill, et al.*, "Osteoporosis: A possible Autoimmune Etiology", *Ann. of Clin. Lab. Sci.*, Vol. 17, pgs. 255-256, (1987)). The concept of a lymphocyte-derived alkaline phosphatase is further described by *Griffiths, et al.* See *Griffiths, J., et al.*, "Separation and Identification of Alkaline Phosphatase Isoenzymes and Isoforms in Serum of Healthy Persons by Isoelectric Focusing", *Clin Chem*, Vol. 32, pgs. 2171-2177, (1987). Thus, osteoporosis is well suited to diagnosis by interpretation of laboratory data.

Osteoporosis poses a major health problem in the United States, not only for those persons who are already affected but for individuals whose diet, life style, and body build make them more likely to develop osteoporosis as they age. Postmenopausal osteoporosis is a common disorder that results in substantial morbidity and mortality. As many as 25 million American women suffer from osteoporosis. Osteoporosis is responsible for approximately 250,000 femoral neck fractures annually in the United States, and these fractures are associated with a 20% mortality rate within 6 months. The risk is particularly high among the elderly, who also tend to lose bone as a result of the aging process. *Wu K., et al.*, "Bone Resorption Rates in Physiological, Senile, and Postmenopausal Osteoporosis." *J. Lab Clin. Med.*, Vol. 69, pg. 810, (1967).

It is estimated from a survey of medical clinics that of those individuals living to age 85, 32% of the women and 17% of men will fracture hips, weakened by osteoporosis. In addition to the pain and suffering caused by these fractures, the monetary cost is great, accounting for well over 3.8 billion dollars per year in treatment for fractures of osteoporosis. Moreover, six to eight months following hip fracture, about 50% of the osteoporotic patients are in need of assistance with activities of daily living and about 25% require nursing home care. Only about 25% of the patients fully recover. In view of the costs associated with osteoporosis, as measured in dollars and in human suffering, osteoporosis has increasingly been perceived as a serious and disabling disease, warranting substantial involvement on the part of clinical investigators, governmental agencies, and pharmaceutical industries to develop and evaluate potential treatments and early detection techniques.

The National Institutes of Health 1984 Consensus Conference stimulated those interested in bone density with its recommendations for "defining persons at risk, and developing safe, effective and low cost strategies for fracture protection." See, Office of Medical Applications of Research National Institutes of Health, "Osteoporosis: consensus conference," *JAMA*, Vol. 252, pgs. 799-802, (1984). Since 1984, there has been a dramatic change in the way physicians view osteopenia. With the development and popularization of highly sensitive techniques such as DPA and quantitative computed tomography, physicians are now capable of measuring the density of the proximal femur and the lumbar vertebrae. (For a general review of the methods currently available to measure bone density, see *Avioli, L.V., ed.*, "Metabolic Bone Disease and Clinically Related Disorders", W.B. Saunders Company (1990)).

An early decrease in bone mass can be measured by non-invasive assessment of the skeleton by four widely available methods, including single photon absorptometry, dual photon absorptometry, dual-energy x-ray absorptometry, and quantitative computed tomography. A brief discussion of these diagnostic methods follows.

A device called a single-photon absorptometer (SPA) is used to measure bone mineral content, primarily in the forearm and wrist. The heel bone can also measured using SPA because the heel bone is thought to be a predictor of bone loss in the spine. SPA measures primarily cortical bone,

which is also affected by osteoporosis, though not to the same extent as trabecular bone.

The technique of dual-photon absorptometry (DPA) provides a measurement of the total cortical and trabecular mineral content of the hip and spine. DPA uses less radiation than conventional x-rays; but, a scan of the spine using DPA still exposes the patient to approximately one tenth of the radiation that results from a routine chest x-ray.

Dual-energy x-ray absorptometry (DXA) provides a measurement of the amount of bone tissue in the hip and spine. This technique is now used routinely because it is faster than DPA.

Unlike DPA and DXA, quantitative computed tomography, more commonly called a CAT scan, can measure the density of either bone or just the trabecular portion. CAT scans unfortunately expose patients to higher doses of radiation than any of the other techniques.

Radiographic absorptometry (RA) is a method for non-invasive measurement of bone mineral x-rays of the hand. Radiographs, taken with a standard x-ray machine, are sent to a central laboratory for computer-controlled analysis.

As osteopenia progresses, both the number and thickness of the trabecular units decrease, causing fragility of bone and an increased risk of fractures. The radiographic manifestations of osteoporosis reflect the deficiency of the organic matrix and parallel the gross pathologic findings. The cortices are thin and the trabeculae fine and sparse; the skeletal structures are therefore more radiolucent than normal. The disease process eventually affects almost all of the skeletal structures. The principal areas of demineralization are the spine and pelvis, especially in the femoral neck and head. Demineralization is less marked in the skull and extremities. Although useful to detect breaks in the bone, ordinary x-rays are not sensitive enough to detect osteoporosis until a large amount of bone tissue has already been lost, generally from 25% to 40%. By the time osteoporosis can be identified by x-ray techniques, the condition is advanced.

Current standard diagnostic techniques, are not effective for early detection of osteoporosis. Changes seen in osteoporosis are very gradual, and often go undetected in the early stages of the disease. Osteoporosis is often not detected in its early stages because bone mass must be decreased

by about 30% to 40% before it is apparent using standard x-ray diagnostic techniques. Preventing osteoporosis by detecting early bone loss is far better than identifying the disease at relatively advanced stages and subsequently attempting to prevent its progression. Once major deterioration has occurred and gaps exist between the ends of fractured trabecular, no current treatment can be expected to restore the lost bone. Thus, therapeutic efforts must be directed toward prevention and early recognition of the progressive disease so treatment can be instituted before essentially irreversible structural damage ensues. *Cummings S.R., et al.*, "Should Perimenopausal Women Be Screened for Osteoporosis?", *Ann. Int. Med.*, Vol. 104, pgs. 745-751, (1986); Courpron P., "Bone Tissue Mechanisms Underlying Osteoporosis," *Orthop. Clin. North Am.*, Vol. 12, pg. 513, (1981); *Frost H. M.*, "Mechanical Determinants of Bone Modeling," *J. Metabol. Bone. Dis. Rel. Res.*, Vol. 4, pg. 217, (1983).

One of the problems with the current methods for diagnosing osteoporosis is that the procedures do not give any information about the underlying cause of the osteoporosis, making it difficult to prescribe an appropriate course of treatment for the patient. For example, a common cause of postmenopausal osteoporosis is an estrogen deficit, which x-ray techniques cannot measure. Another problem inherent in the current diagnostic methods for osteopenia is that all of the current methods require expensive, sophisticated medical instrumentation to perform the bone density measurements. Additionally, patients must be exposed to x-rays. This makes a general screening of high risk populations impractical due to the expense and unavailability of the necessary instrumentation to the average clinic.

In view of the difficulties associated with extracting a diagnosis from the laboratory data for a set of predictive biomarkers, there is need for automated diagnostic systems that are capable of complex pattern recognition. There have been several attempts at using computational models to achieve pattern recognition in diagnostics. One of the most popular computational method for making diagnoses from multivariate laboratory data has been discriminate function analysis. However, diagnostic systems that rely exclusively on classical pattern recognition technology (geometric, syntactic, template, statistical) are not effective for evaluating the characteristic biomarker patterns of many disease states.

There is no clear set of rules that accurately describes how to analyze a set of biomarkers to reach a diagnosis.

In recent years, neural networks have been gaining popularity as a means for recognizing and analyzing subtle diagnostic patterns in multivariate laboratory data. Neural networks possess the ability to discern patterns and trends too subtle or too complex for humans and conventional computational methods to identify. While humans can't easily assimilate more than two or three variables at once, neural networks can perceive correlations among hundreds of variables. Examples of areas in which neural networks have been explored for their value in clinical diagnosis and/or prognosis include:

- psychiatry (See *Mulsant, B.H.*, "A Neural Network as an Approach to Clinical Diagnosis", *MD Computing*, Vol. 7, pp. 25-36 (1990));
- autism (See *Cohen, I., et al.*, "Diagnosing Autism: A Neural Net-Based Tool", PCAI, pp. 22-25 (May/June 1994);
- pediatric radiology (See *Boone, L.M., et al.*, "Neural Networks in Radiologic Diagnosis. I. Introduction and Illustration", *Invest. Radiol.*, Vol. 25, pp. 1012-1016, (1990) and *Gross, G.W., et al.*, "Neural Networks in Radiologic Diagnosis. II. Interpretation of Neonatal Chest Radiographs", *Invest. Radiol.*, Vol. 25, pp. 1017-1023 (1990));
- breast cancer (See *Astion, M.L., et al.*, "Application of Neural Networks to the Interpretation of Laboratory Data in Cancer Diagnosis", *Clin. Chem.*, Vol. 38, No. 1, pp. 34-38 (1992); *Yuzheng, W., et al.*, "Artificial Neural Networks in Mammography: Application to Decision Making in the Diagnosis of Breast Cancer", *Radiology*, Vol. 82, pp. 81-87 (1993); *Kappen, H.J., et al.*, "Neural Network Analysis to Predict Treatment Outcome", *Annals of Oncology*, Vol. 4, Supp. 4, pp. S31-S34 (1993); and, *Ravdin, P.M., et al.*, " A practical application of neural network analysis for predicting outcome of individual breast cancer patients", *Breast Cancer Research and Treatment*, Vol. 22, pp. 285-293 (1992));
- ovarian cancer (See *Wilding, P., et. al.*, "Application of backpropogation neural networks to diagnosis of breast and ovarian cancer", Cancer Letters, Vol. 77, pp. 145-153 (1994)).

- thyroid disease (See, *Sharpe, P.K., et. al.*; "Artifical Neural Networks in Diagnosis of Thyroid Function from in Vitro Laboratory Tests," *Clin. Chem.*, Vol. 39, No. 11, pps. 2248-2253 (1993)).
- cervical cancer (See U. S. Patent No. 4,965,725 to *Rutenberg*); and,
- cardiology (See U.S. Patent No. 5,280,792 to *Leong et al.* and *Furlong, J.W.*, "Neural Network of Serial Cardiac Enzyme Data: A Clinical Application of Artifical Machine Intelligence", *Clin. Chem.*, Vol. 96, No. 1, pp. 134-141 (July 1991).

Neural networks are capable of pattern recognition particularly suited to making diagnoses. Unlike current methods for arriving at a diagnosis from a logical set of rules, neural networks do not require explicit encoding of process knowledge in a set of rules. Neural networks learn from examples.

The present invention provides a new method for diagnosing diseases and screening patients at risk for a disease, utilizing laboratory data to obtain a diagnostic index. It further provides a method for early detection and diagnosis of a disease. As applied to osteoporosis, it generates a new level of interest in screening individuals who are at risk for osteoporosis because this novel diagnostic procedure provides information about the underlying cause of the osteopenia. The present invention also provides a simple, inexpensive, and rapid method of estimating bone density using serum blood levels of specific, predetermined blood constituents. As applied to cancer, it provides a simple, inexpensive, accurate and rapid method for detecting cancer, resulting in earlier and less invasive treatment.

Summary of the Invention

The present invention is directed to an *in vitro*, biological fluid-based method for diagnosing, screening or prognosing for diseases in which laboratory data are manipulated to obtain a diagnostic index. The term "biological fluid" includes, but is not limited to, blood, urine, saliva, cerebral spinal fluid, synovial fluid, and tears. In the preferred embodiments of the present invention, the method of the present invention is used to provide a of cancer and osteoporosis.

The present invention is a method for diagnosing, screening or prognosing a disease in a human or animal comprising the steps of

measuring the concentrations of a predetermined set of biomarkers known to be associated with the disease from a biological fluid from the human or animal; scaling the digitized values of the analytes; and sending the scaled values to a trained neural network, whereby the output values from the neural network tend toward the upper value when the human or animal has the disease and the output values tend toward the lower value when the human or animal does not have the disease.

The present invention also comprises an apparatus for diagnosing, screening or prognosing a disease in a human or animal comprising a means for digitizing the concentrations of a predetermined set of biomarkers known to be associated with the disease from a biological fluid from the human or animal; a means for scaling the digitized values; and a trained neural network coupled to the digitizing and scaling means for generating network output values between an upper and lower value, whereby the output values tend toward the upper value when the human or animal has the disease and the output values tend toward the lower value when the human or animal does not have the disease.

The present invention also comprises a method for diagnosing, screening or prognosing a disease in a human or animal comprising the steps of measuring the concentrations of a predetermined set of biomarkers known to be associated with the disease from a biological fluid from the human or animal, scaling the digitized values of the analytes, and introducing the scaled values to a first trained neural network, whereby the output values from the first neural network tend toward the upper value when the human or animal has the disease and the output values tend toward the lower value when the human or animal does not have the disease; and sending the output value from the first neural network and a second set of predetermined biomarkers, which could include one or more of the biomarkers in the first set of predetermined biomarkers, to a second trained neural network, whereby the output values from the second neural network tend toward the upper value when the human or animal has the disease and the output values from the second neural network tend toward the lower value when the human or animal does not have the disease.

In accordance with the first embodiment of the present invention, a trained neural network is utilized to determine a diagnostic index corresponding to the presence and severity of a disease by analyzing a set of

predetermined biomarkers for that disease. In accordance with the invention, the concentrations, or in some cases, the presence of certain biomarkers related to the incidence of a particular disease are determined for a patient. These values are then scaled and the scaled values are then sent to a trained neural network to yield a diagnostic index. A neural network is trained by introducing a population of patients in which a disease state is known, along with the biomarker values for those patients and "teaching" the neural network to recognize the patterns in the biomarkers. After the neural network is trained, biomarker values from patients with unknown disease states are introduced to the trained neural network. The neural network then processes the information to produce a value corresponding to a diagnosis of the presence or absence and the severity of a particular disease.

The inventors propose that the artificial neural network, especially the multi-layer feedforward network, may, through their weight connections, correspond to biomarker patterns that are important for categorizing diseases. Additionally, the inventors propose that the neural network is able to identify unique patterns of biomarkers associated with a variety of disorders that may help to classify borderline cases that do not appear to fit into either a malignant or benign pattern.

A second embodiment of the present invention involves a two step analysis of the biomarkers by neural network. This avoids the bias created by a dominant predictive variable when training a network. The dominant biomarker or predictive variable is excluded from the first analysis by neural network and then included in a second analysis by neural network. For example, if age is thought to be the dominant predictive variable in the diagnosis of osteoporosis, that variable is not included in the training of the first neural network, and the training data set is limited to the other selected biomarkers. After obtaining a diagnostic index using the first set of biomarkers, a second neural network is trained using the diagnostic index and the entire set of input variables, including age, to yield another diagnostic index. The final diagnostic index is a composition of an artificial neural network generated index and results from heuristic analysis using other non-numerical patient information.

The present invention further comprises an apparatus capable of diagnosing, screening or prognosing a disease comprising a sample

receiving means, a sample detecting means capable of detecting the quantity of one or more biomarkers in a biological fluid, and, after digitalizing the quantity of analyte in the biological fluid, scaling the digitized values and introducing the scaled data into a neural network which yields a therapeutic index on a printer or, optionally, on a video display.

A third embodiment of the present invention includes a method for diagnosing and determining the severity and underlying cause of osteopenia using blood concentrations of a specific, predetermined set of blood constituents. The method comprises the steps of (1) measuring the severity of the disease in a set of humans or animals with varying severity of disease by a standard method, (2) assigning the severity of disease a numerical value on a severity scale, the scale being from no disease to severe disease. (3) measuring the blood concentrations of the predetermined set of blood constituents in the set of humans or animals with varying severity of the disease, and then (4) calculating a numerical relationship between the set of blood concentrations and the severity of disease.

The present invention also comprises a simple and rapid method of determining severity of osteopenia in a patient. In a preferred embodiment, the method comprises determining the serum level of the following serum constituents: calcium, phosphate, total alkaline phosphatase, an alkaline phosphatase isoenzyme, estradiol, and progesterone. The alkaline phosphatase isoenzyme is preferably t-lymphocyte derived alkaline phosphatase or blood, liver or intestinal alkaline phosphatase isoenzyme. The results of these tests are then introduced into an algorithm. Optionally, the age of the patient may also be factored into the equation. The bone density coefficient that is calculated by the algorithm correlates to a very high degree to bone density as measured by standard methods, such as radiographic absorptometry, quantitative computed tomography, dual photon absorptometry and direct measurement of bone density. The bone density coefficient that is measured is then compared to an osteopenic severity scale.

Using the six serum constituent concentrations, the present invention can be used to determine the osteopenic state of a patient as well as give an indication of the underlying cause of the osteopenia. The present invention can be correlated to any method of measuring bone density simply by

recalculating the coefficients in the algorithm using a multiple linear regression analysis.

Accordingly, it is an object of the present invention is to provide a method for diagnosing, screening or prognosing and determining the severity of a disease using the concentrations and values of a predetermined set of biomarkers.

It is a further object of the present invention to provide a more accurate method for screening, prognosing and diagnosing breast cancer using biological fluid-based laboratory data.

It is another object of the present invention to provide a more accurate method for screening and diagnosing ovarian cancer using biological fluid-based laboratory data.

Yet another object of the present invention is to provide a more accurate method for screening, prognosing and diagnosing colon cancer using biological fluid-based laboratory data.

Furthermore, it is an object of the present invention to provide a more accurate method for screening, prognosing and diagnosing prostate cancer using biological fluid-based laboratory data.

Additionally, it is an object of the present invention to provide a more accurate method for screening, prognosing and diagnosing testicular cancer using biological fluid-based laboratory data.

Another object of the present invention is to provide a method for diagnosing cancer which will provide a better understanding of the probable cause of the cancer.

Another object of the present invention is to provide a diagnostic test for cancer which can be used to screen large numbers of individuals.

Another object of the present invention is to provide a simple and rapid chemical test for diagnosing osteoporosis.

Another object of the present invention is to provide a test for osteoporosis which will also give information as to the underlying cause of the osteopenic condition.

Another object of the present invention is to provide a diagnostic test for osteoporosis which can be used to screen large numbers of individuals.

Yet another object of the present invention is to provide a method for diagnosing osteoporosis and determining the underlying cause of the osteopenia without having to subject the patient to radiation.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiment and the appended claims.

Brief Description of the Figures

Fig. 1 illustrates a feed forward neural network having multiple outputs.

Fig. 2 illustrates a feed forward neural network having a single output.

Fig. 3 is an equation illustrating the mathematical relationship between the input and output of a neuron.

Fig. 4 is a schematic illustration of the second preferred embodiment of the present invention.

Fig. 5 is a graph showing the change in the neural network diagnostic index obtained by the method of the present invention over time for a ovarian cancer patirent.

Fig. 6 is a graph showing the change in the neural network diagnostic index obtained by the method of the present invention over time for a prostate cancer patirent.

Fig. 7 is a graph showing the change in the neural network diagnostic index obtained by the method of the present invention over time for a colon cancer patirent.

Fig. 8 is a graph showing the change in the neural network diagnostic index obtained by the method of the present invention over time for a breast cancer patient.

Fig. 9 shows the patient data used to develop and test the osteoporosis neural network diagnostic system.

Fig. 10 shows the patient data used to test the osteoporosis neural network diagnostic system.

Fig. 11 is a program list of the algorithm of the third embodiment of the present invention.

Fig. 12 shows the discrepancies between the DPA measurements and the measurements obtained using the method of the present invention for individual subjects.

Fig. 13 shows the correlation of the results obtained using the present invention and DPA.

Fig. 14 shows the normality of values predicted by the present invention.

Fig. 15 shows the normality of residuals (differences between DPA measurements and values predicted according to the method of the present invention), as evidenced by the arrangement of points along a diagonal straight line.

Fig. 16 shows that the variance of residuals.

Fig. 17 shows the independence of residuals.

Fig. 18 shows that virtually all observed DPA measurements are predicted by the method of the present invention.

Fig. 19 shows the training data used to construct the prostate cancer neural network prognostic system.

Fig. 20 shows the relationships between the input neurons, hidden layer and output neuron of a neural network in one embodiment of the present invention.

Detailed Description

The first embodiment of the present invention is directed to an in vitro, serum-based computer assisted method for screening, prognosing and diagnosing diseases utilizing a neural network to obtain a conclusive diagnosis. The present invention can be adapted to existing diagnostic devices that have a collection means, a sample detecting means capable of detecting the quantity of an analyte in a biological fluid and a means of either printing or displaying the results of the tests on video display means.

The inventors have discovered that biomarkers collectively alter in response to a disease process, and collectively constitute a new diagnostic biomarker with better disease predictability than the individual biomarkers. When the biomarkers are processed and analyzed as a group to yield a single diagnostic index, the sensitivity and specificity of the diagnosis is increased, making it possible for a physician to detect the presence of a disease earlier and with greater precision, or estimate a prognosis with greater precision, than by analysis of the individual biomarkers.

In accordance with the method of this invention, blood is first collected from a patient, and then centrifuged to separate the red blood cells from the serum. It should be understood that biological fluids other than blood can be used in practicing the present invention. Next, the serum is

analyzed, using standard laboratory techniques, to determine the concentrations, or in some cases the presence or absence, of specific predetermined biomarkers related to the incidence of a particular disease. It is to be understood that this process can be carried out automatically in conventional diagnostic machines. For purposes of illustration, a description of the methods for obtaining the values for the biomarkers for osteopenia is provided elsewhere in this section.

The biomarkers relied upon to diagnose a disease by the method of the present invention must be predictive of the suspected disease and must be statistically significant for analysis by a neural network. The selection of biomarkers that offers statistically significant discriminating power in the diagnosis of disease involves several steps. First an inventory of biomarkers that have shown certain relevancy in the diagnosis of the disease of interest must be conducted. In general, only the biomarkers that reflect different aspects of the disease process or other diagnostic information need to be included. Second, the selected biomarkers need to have a reasonable diagnostic value in terms of sensitivity, specificity, and positive and negative predictive powers. The design and implementation of experimental protocol from which the biomarkers are developed and evaluated should also be considered. Third, if the number of candidate biomarkers is large, a formal discriminating power analysis may be conducted. However, many of the standard statistical analysis methods may not be adequate for highly nonlinear classification problems. If the number of candidates are not too large, they may be all included in the initial attempt of neural network training. If one or several of the input biomarkers to the network are irrelevant to the classification decision making process, it will be reflected in the network connection weights of the trained neural networks. These values may then be removed from the biomarker set for a particular disease. Other methods for evaluating the statistical significance of a biomarker selected for analysis by neural network and selecting biomarkers for training a neural network are well known in the art.

Biomarkers which meet the criteria delineated above, namely, they are predictive of a particular disease and statistically significant for analysis by neural network, are identified below for a sample of diseases including ovarian cancer, prostate cancer, colon cancer, breast cancer, testicular cancer and osteoporosis.

Ovarian Cancer	Prostate Cancer I	Colon Cancer
LASA-P®	LASA-P®	LASA-P®
CA125	PAP	CA 19-9
DM/70K	PSA	CEA

Prostate Cancer II
PAP
PSA
CK-BB
Acid Phosphatase

Breast Cancer	Testicular Cancer	Osteoporosis
LASA-P®	LASA-P®	Calcium
CEA	AFP	Phosphate
HER2/neu in Plasma	HCG-Beta	Estradiol
	CA 15-3®	Progesterone
		ALP
		ALP Isoenzyme 1
		ALP Isoenzyme 2

A key to the abbreviations used above is provided below:

AFP:	Alpha-Fetoprotein
CA125:	Cancer Antigen 125
CA 15-3®**	Breast Antigens 115D8/DF3
CA 19-9:	Carbohydrate Antigen 19-9
CEA:	Carcinoembryonic Antigen
CK-BB:	Creatinine kinase, BB subfraction
DM/70K:	Ovarian marker NB/70K
HCG-Beta:	Human Chorionic Gonadotropin, Beta Sub-Unit
HER 2/neu in Plasma:	c-erb B-2 (HER2/neu) oncoprotein in plasma
LASA-P®*:	Lipid-Associated Sialic Acid in Plasma
PAP:	Prostatic Acid Phosphatase
PSA:	Prostate Specific Antigen

*LASA-P is a registered trademark of DIANON Systems, Inc.

**CA 15-3 is a registered trademark of Centocor, Inc.

A wide number of diseases may be diagnosed in accordance with the method of the present invention. To be suitable for diagnosis by the present method, biomarkers for the disease must be quantifiable. The biomarkers must also be predictive of the disease and must be statistically significant relative to one another. The method of the present invention is equally suited to the diagnosis of any disease in which serum biomarkers can be identified, including but not limited to infectious diseases, and genetic abnormalities.

After determining the serum biomarkers for a disease, the biomarker values are analyzed by a trained neural network to yield a single diagnostic value. The most common neural network architecture for pattern classification problems is the feedforward network, which typically consists of an input layer, one or more hidden layers, and an output layer. Figs. 1 and 2 illustrate the arrangement of neurons in two different feedforward networks.

The elements that make up each layer of a neural network are referred to as neurons or nodes. Inputs are fed forward from the input layer to the hidden layers and then to the output layer. The number of neurons in each layer is determined before the network is trained. Typically, there is one input neuron or node for each input variable, and one output node for each output. The inputs to the neural network are predictor variables. These predictor variables can be quantitative or qualitative. Neural networks make no data distribution assumptions and can simultaneously use both quantitative and qualitative inputs. In the present invention, the biomarker values, after a linear prescaling to values between 0.0 and 0.1, constitute the input variables.

The outputs of the network represent output categories. For example, a malignancy may be represented by maximal output of the malignant output neuron and silence of the benign neuron, whereas a benign process is represented by maximal output of the benign neuron and silence of the malignant neuron. A simple arithmetic function combines outputs of the two neurons to yield a single diagnostic index. In the alternative, a single output neuron may be used. An output of greater than 0.5 would indicate a malignancy and an output of less than 0.5 would indicate a benign condition. In this way a diagnostic index is directly obtained.

The number of hidden layers and the number of nodes in the hidden layers are configurable parameters that have a significant influence on the performance of the network. In practice, the optimal number of hidden neurons is determined empirically. The means for determining the optimum numbers of hidden neurons is well known to those skilled in the art and depends on the complexity of the problem being solved.

In the present invention, the main network model is a multi-layer feedforward perceptron using a backpropogation training algorithm. The number of hidden layers and the number of neurons in each hidden layer was determined to adequately match the level of complexity of the diagnostic problem. With the assumption that the samples in the training set are representative of all possible situations encountered in real applicationswith no significant contradictions, and and the number and stratification of samples in the generalization test are statistically adequeate, the criteria outlined below is used to determine if a chosen network configuration is appropriate.

If the network continues to fail to correctly classify large portions of the samples in the training set, even after many adjustments of training algorithm parameters, the network complexity should be increased.

On the other hand, if the the network acheives a high rate of correctly classifying the training set but fails to accurately classify a large number of samples in the testing set, network structure is probably too complex for the problem being solved, i.e. it has sufficient inherent flexibility to fit the training data set, but not sufficient predictive power to classify the test data set. If this is the case, the number of neurons in the hidden layers should gradually be reduced, or, if there are mutiple hidden layers, the hidden layers should be gradually reduced.

Note that it is not always necessary to have a large training sample set. If the samples in a training set have already represented all possible cases with adequate statistical significance, the addition of new samples genewrally does not increase the amount of information in the training samples. Instead it may decrease the useful information to nosie ratio in the samples. At the other extreme, too small a training set will generally not be able to cover all possible variations in the population. The resultant network often simply memorizes all the cases in the training set and does not generalize at all.

The input and output layers are not directly connected. Every input neuron is connected to every neuron in the following hidden layer and neuron in ahidden layer is connected to every neuron in the following adjacent hidden layer or output layer, depending on the number of hidden layers. Each of the multiple connections to a particular neuron is weighted. In the hidden and output layers, each node sums the input activations, multiplied by the respective connection weights plus a bias term. The weighted sum then passes through a non-linear output function, typically a sigmoidal function, which gives the network the ability to represent complex non-linear relationships. A neuron fires if the sum of the weighted inputs to it are greater than a threshold value. As illustrated in Fig. 3, once a neuron is above a threshold, the magnitude of its output is a sigmoid function of the net input. The end result of activity in the neural network is the net output, a complex nonlinear function of the inputs.

In summary and in accordance with the present invention, first the values of the biomarkers for a specific disease are determined and scaled. The biomarkers are fed forward from the input layer to the hidden layer (or layers) and then to the output layer of the neural network. The number of neurons in the input layer is determined before the network is trained and correspond to the number of biomarkers predictive for a specific disease. The biomarkers are preselected. There is one input neuron for each diagnostic variable or biomarker, and one output neuron for each desired output. Other than the identified biomarkers, diagnostic variables may include demographic information such as the age, race or sex of the patient and whether the patient is pre- or post-menopausal. The number of neurons in the output layer depends on the type of output desired. The number of neurons in the hidden layer is determined euperically during training.

The neural network used for diagnosing a specific disease must be trained to do so. In accordance with the present invention, the neural network is trained by back propagation. Back propagation refers to the technique of training a neural network to accurately model a set of input and output examples by determining the best connection weights between the values, and is well known in the art. Other techniques which may be used to train a neural network for purposes of this invention may include any other non-linear global optimization technique, such as the genetic search

algorithm; however, the feed forward, back propogation network is most popular.

At the beginning of training, the connection weights in the network are randomly initialized. The training data is then presented to the network one at a time. In accordance with the present invention, the training data consists of the biomarker values for a group of patients, and the diagnosis for each of those patients. The biomarker values are the input variables used to train the network. For each patient, the network uses the patient's biomarker values to estimate a diagnosis, which is then compared to the actual diagnosis. If the network's diagnosis is correct, then the connection strengths and thresholds within the network are not changed, and the next patient is presented to the network. If the estimate of the diagnosis is not correct, the connection weights and thresholds in both the hidden layer and the output layer are adjusted to reduce the size of the classification error. After adjustments are made, the next patient is presented. Training proceeds until all patients in the training group are correctly classified. If the network is not able to completely classify all the data it will train indefinitely, in which case training is terminated.

When training the neural network, the trainer may set the decision limits regarding the definition of a classification error, i.e. an incorrect diagnosis. The relevant parameter is the error tolerance, which specifies how close the estimated output has to be to the actual output to be correct. For example, if two output neurons are used and the training tolerance is set at 5%, the estimate of malignancy is considered correct if the malignant output neuron fires at 95% of maximum and the benign neuron fires at 5% of maximum. Similarly, a correct estimate of a benign diagnosis means that the benign output neuron firs at 95% of maximum, while the malignant neuron fires at 5% of maximum. The methods for determining a classification error are well known to those skilled in the art.

In the preferred embodiment of this invention, if a single output neuron is used, a benign normal diagnosis is set at an output of 0.1 and a malignant or abnormal diagnosis is set at an output of 0.9. Error tolerance is an adjustable parameter and is significant in determining the success of the network at making an accurate diagnosis. It is preferred to use either one or two output neurons.

After the neural network is trained for the desired disease, biomarker values from patients with unknown disease conditions are introduced to the trained neural network. The neural network then processes the information to produce a value corresponding to a diagnosis of the presence or absence of the particular disease. In accordance with the present invention, this is accomplished by using either one single output neuron or two output neurons. If two output neurons are used, the output from the two neurons are combined to generate a single diagnostic index.

As illustarted by Fig. 4, in a second embodiment of the present invention, the diagnostic value obtained by analysis of the biomarkers by a trained neural network is further analyzed by a set of heuristic rules in combination with additional patient information. The additional patient information includes things such as family medical history and demographic information. This data is then processed to yield a second single diagnostic value.

This invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof, which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

In the following examples which utilize a neural network in the analysis of the data, a Neuralshell 2, Release 1.5 (Ward Systems Group, Inc.) neural network development program was used for the training of the neural network on a Pentium 60 mhz computer (Magitronic, Inc.).

Example 1

The following example describes the training of a neural network to diagnose colon cancer.

A total of 167 patients (48 with diagnosed colon cancer, and 119 normal) were divided into 2 groups, a training set and a generalization testing set. The training set contained approximately 60% of the patients (90 patients, 27 with diagnosed colon cance, and 63 normal), and the generalization testing set contained approximately 40% of the patients (77 patients, 23 with diagnosed colon cancer, and 54 normal).

The initial network architecture was selected based on the level of complextity of the classification task. A multi-layer feedforward network was used. Selection of the initial architecture involved the selection of the number of hidden layers and the number of neurons in each hidden layer. Several trial iterations were performed to determine and adequeate configuration that showed good results on both the training sample set and the generalization test sample set. This particular neural network had 5 input neurons, one hidden layer having 10 neurons and one output neuron.

Initially, connection weights among the neurons were randomly set. The neural network had five input neurons, corresponding to five input variables for diagnosing colon cancer: LASA-P®; CA 19-9, CEA, Age, and Sex. During training, the five input variables for each patient were first linearly scaled into the continuos range between 0.0 and 1.0. The resultant five numbers were then presented as an input vector to the input neurons of the artificial neural network.

For each of the input vectors, the network generated an output based on the connection weights among the network neurons. The output can be a single value or a vector of numbers, depending on the number of output neurons used. Each neuron in the network participated in the output calculation by passing the sum of all inputs to the neuron through a non-linear s-shaped function (often a ligistic function) and sending the result to each and everyone of the neurons in the following adjacent layer. The generated output was compared to the desired "target" output. A value of 0.1 corresponded to a diagnosis of normal and an output of 0.9 corresponded to a diagnosis of of abnormal. The difference was used to calculate an error term to guide the training algorithm, i.e., the backpropogation algorithm, in the adjustment of network connection weights in an attempt to reduce the differences between network outputs and target values over the training sample set.

To counteract over learning, which can occur when a neural network is too large for the problem and trains too long, the network was tested on the generalization test set every 200 iterations. The networks for which generalization testing showed reduced root mean squared error over previous tests were saved and used in later calculations or generalizations. This procedure was repeated until generalization failed to improve over 2000 successive tests.

To accelerate the rate of solution during the initial stage of training, the learning rate for the hidden layer was initially set at 0.1 and momentum was set at 0.05, for the first several iterations. After the first several iterations, the learning rate was lowered to 0.01 and momentum to 0.0005 to narrow in on the appropriate solution.

After training, the neural network correctly identified 95% of the patients without cancer in the training data set, and 92% of the patients with cancer.

When presented with the generalization test results, the trained neural network correctly identified 100% of the people with normal diagnosis and 95% of the people with cancer diagnosis.

Example 2

The following example illustrates the value of the present invention for diagnosing ovarian cancer.

The biomarker values for LASA-P®, CA 125, and DM/70K were measured in a patient suspected of having ovarian cancer. Two of the biomarker values fell within the normal reference range, and one value fell outside the normal reference range. The values were scaled and analyzed by a trained neural network, yielding a diagnostic index of 75, indicating the presence of ovarian cancer. The diagnotic index generated by the neural network (between 0.1 and 0.9) is multiplied by 100 to obtain a whole number. The normal reference range for the diagnostic index was 0-50. The biomarker values measured, together with the normal reference range for those values are provided below:

Biomarker	Patient Value	Normal Reference Range
LASA-P®	18	15-20 mg/dl
CA 125	16	0-35 U/ml
DM/70K	45	0-35 U/ml
Neural Net Diagnostic Index	75	0-50

The patient underwent surgery to treat the cancer. Fig. 5 graphically illustrates the change in the neural network diagnostic index over time, before and after surgery.

Example 3

The following example illustrates the value of the present invention for diagnosing prostate cancer.

The biomarker values for LASA-P®, PAP, and PSA were measured in a patient suspected of having prostate cancer. All three of the biomarker values fell within the normal reference range. The values were scaled and analyzed by a trained neural network, yielding a diagnostic index of 90, indicating the presence of prostate cancer. The normal reference range for the diagnostic index was 0-50. The biomarker values measured, together with the normal reference range for those values are provided below

Biomarker	Patient Value	Normal Reference Range
LASA-P®	16	15-20 mg/dl
PAP	1.5	0-2.5 ng/ml
PSA	1.0	0-2.5 ng/ml
Neural Net Diagnostic Index	90	0-50

The patient underwent surgery to treat the cancer. Fig. 6 graphically illustrates the change in the neural network diagnostic index over time, before and after surgery.

Example 4

The following example illustrates the value of the present invention for diagnosing colon cancer.

The biomarker values for LASA-P®, CA 19-9, and CEA were measured in a patient suspected of having colon cancer. All three of the biomarker values fell within the normal reference range. The values were scaled and analyzed by a trained neural network, yielding a diagnostic index of 93, indicating the presence of colon cancer. The normal reference range for the diagnostic index was 0-50. The biomarker values measured, together with the normal reference range for those values are provided below:

Biomarker	Patient Value	Normal Reference Range
LASA-P®	17.9	15-20 mg/dl
CA19-9	5.0	0-37 U/ml
CEA	2.3	0-2.5 ng/ml
Neural Net Diagnostic Index	93	0-50

The patient underwent surgery to treat the cancer. Fig. 7 graphically illustrates the change in the neural network diagnostic index over time, before and after surgery.

Example 5

The following example illustrates the value of the present invention for diagnosing breast cancer.

The biomarker values for LASA-P®, CEA, HER2/neu in Plasma and CA 15-3® were measured in a patient suspected of having breast cancer. All four of the biomarker values fell within the normal reference range, although two of the biomarker values were on the high end of the normal range. The values were scaled and analyzed by a trained neural network, yielding a diagnostic index of 91, indicating the presence of breast cancer. The normal reference range for the diagnostic index was 0-50. The biomarker values measured, together with the normal reference range for those values are provided below:

Biomarker	Patient Value	Normal Reference Range
LASA-P®	18	15-20 mg/dl
CEA	1.0	0-2.5 ng/ml
HER2/neu in Plasma	19	0-20 U/ml
CA 15-3®	8	0-30 U/ml
Neural Net Diagnostic Index	91	0-50

The patient underwent surgery to treat the cancer. Fig. 8 graphically illustrates the change in the neural network diagnostic index overtime, before and after surgery.

Example 6

This example illustrates the construction and training of a neural network for diagnosis of ovarian cancer.

Table I below provides the data used to train the network (age, LASA-P®, CA 125, and DM/70K), the actual histological diagnosis, and diagnostic index determined by the neural network. A histological index of 0.1 corresponds to a normal diagnosis and a histological index of 0.9 corresponds to an abnormal diagnosis. A neural net diagnostic index less than .5 corresponds to a normal diagnosis. A neural net diagnostic index greater than .5 corresponds to an abnormal diagnosis.

TABLE I

Training Data for Ovarian Cancer Diagnostic System

Patient Number	Age	LASA-P®	CA 125	DM/70K	Histological Diagnosis	Neural Net Diagnostic Index
94	34	15.8	4	7	0.1	0.225
99	27	19.1	32	7	0.1	0.357
100	33	27	19	27	0.1	0.317
101	32	16.8	7	0	0.1	0.236
102	29	17.5	8	13	0.1	0.186
106	25	18	3	0	0.1	0.137
110	36	17.7	21	0	0.1	0.419
112	37	12.4	10	0	0.1	0.318
114	27	16.2	7	11	0.1	0.159
2001	44	43.5	1880	182	0.9	0.657
2007	51	27	142	86	0.9	0.75
2009	64	20.4	55	32	0.9	0.87
2010	72	27.1	7	43	0.9	0.789
2015	39	22	60	55	0.9	0.568
2019	35	16.5	13	0	0.9	0.327
2020	66	23.1	19	46	0.9	0.79
2022	69	23.2	189	28	0.9	0.905

2024	88	22.4	47	32	0.9	0.962
2026	69	12.6	11	0	0.9	0.807
2027	72	25.3	58	21	0.9	0.923
2031	61	25.1	1480	58	0.9	0.848
2032	79	24.7	78	21	0.9	0.946
2034	65	30.6	439	45	0.9	0.876
2036	48	14.2	13	0	0.9	0.538
2037	72	16.5	15	0	0.9	0.858
2038	68	13.8	25	11	0.9	0.851
2039	69	12.1	10	3	0.9	0.796
2040	79	25.2	50	45	0.9	0.939
46	42	22.6	3	15	0.1	0.34
49	25	16.1	6	7	0.1	0.14
51	28	15.2	17	0	0.1	0.251
52	40	19.1	0	0	0.1	0.303
53	27	25	4	5	0.1	0.17
56	31	24.6	0	0	0.1	0.196
57	33	13.7	7	3	0.1	0.235
58	25	24.8	12	9	0.1	0.19
59	25	25	9	11	0.1	0.169
60	25	17.4	5	0	0.1	0.146
61	35	23.3	0	15	0.1	0.217
62	29	15.4	12	0	0.1	0.229
63	28	18.3	2	1	0.1	0.16
65	33	15.4	8	9	0.1	0.236
68	30	22.8	2	15	0.1	0.171
70	28	15.4	24	1	0.1	0.305
71	31	20	6	19	0.1	0.193
73	30	18	9	0	0.1	0.228
75	35	23.1	1	0	0.1	0.25
76	35	20.4	3	0	0.1	0.258
80	34	18.9	1	6	0.1	0.215
82	32	18.2	72	152	0.1	0.435

84	33	17.4	19	21	0.1	0.305
85	26	15.6	7	5	0.1	0.156
89	26	20.7	12	0	0.1	0.205
92	25	20.7	7	0	0.1	0.164
2042	68	16.4	22	3	0.9	0.852
2043	63	14.5	9	0	0.9	0.733
2044	84	17.6	23	17	0.9	0.931
2046	72	17.8	8	15	0.9	0.813
2047	61	13.4	146	15	0.9	0.852
2048	44	20.8	21	0	0.9	0.567
2049	60	17.8	29	0	0.9	0.822

An evaluation of the training data set (n=61) yielded a sensitivity of 96% and a specificity of 100%.

Table II provides the data used to test the network trained with the data provided in Table I.

TABLE II

Testing Data for Ovarian Cancer Diagnostic System

Patient Number	Age	LASA-P®	CA 125	DM/70K	Histological Diagnosis	Neural Net Diagnostic Index
103	43	14.1	8	15	0.1	0.371
104	28	15.9	11	0	0.1	0.211
105	46	15.9	15	0	0.1	0.529
107	44	23.6	10	26	0.1	0.416
108	32	21.1	9	3	0.1	0.258
109	42	14.1	13	12	0.1	0.407
111	35	19.3	9	6	0.1	0.29
113	39	18.7	4	7	0.1	0.305
115	31	21.8	10	0	0.1	0.259
2002	69	16.4	29	21	0.9	0.869
2003	53	13	11	0	0.9	0.599
2004	58	34.3	80	90	0.9	0.823

2005	64	17.8	76	12	0.9	0.882
2006	47	43.1	630	104	0.9	0.699
2008	56	19	6	35	0.9	0.548
2011	83	14.3	16	4	0.9	0.917
2012	60	24.3	20	38	0.9	0.733
2013	81	21.7	12	106	0.9	0.88
2014	69	33.4	12	39	0.9	0.788
2016	55	17.3	49	5	0.9	0.821
2017	68	19.6	11	0	0.9	0.813
2018	56	17.8	94	3	0.9	0.832
2021	68	12.2	5	0	0.9	0.761
2023	45	15.9	71	1	0.9	0.713
2025	61	17.9	34	5	0.9	0.846
2028	43	17.6	30	0	0.9	0.617
2029	42	16	16	0	0.9	0.47
2030	51	27.7	820	57	0.9	0.75
47	31	21.7	5	0	0.1	0.223
48	39	17.1	3	1	0.1	0.304
50	30	17.2	5	26	0.1	0.16
54	44	21.1	4	0	0.1	0.411
55	26	18.5	1	2	0.1	0.135
64	25	17.2	2	0	0.1	0.131
66	48	19.8	5	1	0.1	0.483
67	27	14.7	3	0	0.1	0.149
69	29	17.2	3	12	0.1	0.158
72	26	20.9	2	4	0.1	0.142
74	31	19.8	3	20	0.1	0.173
77	25	15	10	0	0.1	0.168
78	25	12.4	4	0	0.1	0.132
79	35	20.7	1	6	0.1	0.233
81	26	17.2	9	0	0.1	0.178
83	45	17.1	2	10	0.1	0.372
86	32	15.6	11	0	0.1	0.262

87	31	21.5	6	11	0.1	0.21
88	26	17.2	3	0	0.1	0.145
90	37	16.5	2	2	0.1	0.262
91	30	16.9	7	0	0.1	0.211
93	34	21.7	8	1	0.1	0.285
95	35	20	13	0	0.1	0.339
96	34	22.4	7	3	0.1	0.275
97	37	15.9	6	6	0.1	0.284
98	38	23.5	38	0	0.1	0.624
2033	72	17.6	3	0	0.9	0.806
2035	38	21.6	19	12	0.9	0.422
2041	59	21.5	1660	9	0.9	0.857
2045	61	23	436	46	0.9	0.844
2050	55	28.4	480	139	0.9	0.794

The testing data (n=59) yielded a sensitivity of 92% and specificity of 91%.

The combined results of the training and testing data yielded a sensitivity of 94% and specificity of 96%.

Fig. 20 provides the equations describing the weight matrices between the layers in the neural network

Table III below compares the sensitivity and specificity of a diagnosis obtained by the method of the present invention with the sensitivity and specificity of each individual biomarker. As is evident from the results, the individual biomarkers may be combined by this method to yield a single diagnostic index having greater sensitivity than the individual tests for purposes of diagnosis.

TABLE III

Comparision of Ovarian Cancer Diagnostic Methods

BioMarker Test	Sensitivity	Specificity
Carbohydrate Antigen 125 (CA 125)	74%*	99%*
Dianon Marker 70K (DM/70K)	68%*	99%*
Lipid Associated Sialic Acid (LASA-P®)	79%*	96%*
Neural Network Diagnostic Index for Ovarian Cancer	94%**	96%**

* BioStatistics provided by Dianon Systems, Inc.
** BioStatistics based on initial pilot study performed by Horus Therapeutics, Inc.

Example 7

This example illustrates the construction and training of a neural network for diagnosis of osteoporosis.

Fig. 9 provides the data used to develop, i.e. train and test, the neural network to diagnose osteoporosis. The biomarkers selected included age, calcium, phosphate, estradial (ETWO), progesterone, total alkaline phosphatase, total intestinal alkaline phosphatase, and % liver alkaline phosphatase. Fig. 9 further includes the diagnostic index obtained by the neural network.

Fig. 10 provides the data use to test the network trained with the data in Fig. 10, and the neural network diagnostic index obtained.

The second embodiment of the present invention also relates to a method for diagnosing diseases in humans or animals using blood concentrations of specific, predetermined blood constituents. More specifically, the present invention relates to a method for detecting the occurrence of osteopenia, diagnosing osteoporosis and determining its severity and underlying cause. The present invention also facilitates the periodic monitoring of specific physiological functions which may indicate

the onset of osteopenia and correlates to bone mineral density measurements determined by various standard methods.

In practicing one aspect of the present invention, the severity of disease in a set of humans or animals with varying severity of disease is measured by a standard method or methods. The measurement is then assigned a numerical value corresponding to a severity scale. The scale ranges from humans or animals with no disease, to humans or animals with severe disease. The scale is preferably a numerical scale. For example, one could assign a value which corresponds to normal or slight disease, another value which corresponds to moderate disease and a third value which corresponds to severe disease.

The concentration of a predetermined set of blood constituents in the set of humans or animals with varying severity of disease is then determined. According to the present invention, it is preferable to measure the blood constituents in the same set of humans or animals in which the severity of disease was measured by the conventional method or methods.

After determining the blood concentrations of the predetermined set of blood constituents in the set of humans or animals with varying severity of the disease, mathematical manipulations are performed in which the numerical value obtained using the conventional diagnosis is set to equal the result of a mathematical model utilizing the set of blood constituent measurements. For example, the relationship can be made using a multiple linear regression analysis. It is to be understood that other mathematical models could be used to determine a correlation between the serum constituent concentrations and a standard method of determining severity of the disease. The concept of using mathematical models to determine the relationship or correlation is considered to be part of the present invention. Standard statistical analyses that are well-known to those of ordinary skill in the art are then performed to determined the confidence level of the correlation between the diagnosis by conventional means and the set of blood constituents. These statistical analyses include chi-square tests.

An example of practicing one embodiment of the present invention is a method for diagnosing osteopenia in a human or animal. The method preferably utilizes six blood constituents. These constituents are calcium, phosphate, total alkaline phosphatase, an alkaline phosphatase isoenzyme, estradiol, and progesterone. The alkaline phosphatase isoenzymes preferred

for practicing the present invention include lymphocyte-derived alkaline phosphatase isoenzyme and bone, liver or intestinal alkaline phosphatase isoenzymes. The present invention includes calculating a bone density quotient using the aforementioned six blood constituents by entering the values for the tests into an algorithm that is calculated using a multiple linear regression analysis. Optionally, the age of the patient may also be incorporated into the equation. The bone density coefficient derived from the algorithm allows one to diagnose the osteopenic state of the patient, including the severity of the disease.

In addition to diagnosing the osteopenic state of the human or animal, an indication of the underlying cause of the osteopenia can be determined using the present invention. For example, by practicing the present invention as described herein, one can determine whether the osteopenia in a human or animal is caused by post-menopausal lack of estrogen or is caused by some other condition, such as cancer. This allows the attending physician to be better able to prescribe the appropriate treatment for the osteopenia.

Five of the serum tests that are used in the present invention are tests that are commonly performed by clinical laboratories. The test for t-lymphocyte derived alkaline phosphatase is experimental only; however, the test for blood, liver and intestinal alkaline phosphatase isoenzymes are also known. The type of test used to determine the six serum constituents is not critical to the present invention as long as the tests give accurate blood concentrations of the constituents being measured.

Calcium & Phosphorus:

Maintenance of calcium and phosphorus homeostasis involves the participation of 3 major organs: the small intestine, the kidney, and the skeleton, and is regulated by various hormones. Calcium enters the body through the diet and is absorbed into the circulation from the small intestine. Calcium absorption occurs by two processes, active transport and passive transport. Approximately 98% of the calcium and 85% of the phosphorus in the adult is present in the skeleton primarily as hydroxyapatite, which is a crystal lattice compound of calcium, phosphorus, and hydroxide. Remaining calcium is present in extra cellular fluid, some types of tissue, and skeletal muscle. Phosphorus is combined with lipids, proteins,

carbohydrates, and other organic substances. Of critical importance to calcium homeostasis is the fact that less than 1% of total skeletal reservoir of calcium is rapidly exchangeable with extracellular fluid. In addition to its obvious importance in skeletal mineralization, it is also vital for blood coagulation, neuromolecular condition, maintenance of normal tone and excitability of skeletal and cardiac muscle, and preservation of all membrane integrity and permeability, particularly in terms of sodium and potassium exchange.

Free or ionized calcium accounts for 50% of total calcium. About 5% of total calcium is complexed with a variety of anions, particularly phosphate and citrate. The remaining 45% of calcium is bound to plasma proteins. Both ionized calcium and calcium complexes are freely dialyzable. Acidotic and alkalotic conditions adversely affect the ionized calcium level in the blood. In metabolic bone disease such as hyper- or hypoparathyroidism, Pagets of bone, Vitamin D deficiency, and renal osteodystrophy, calcium and/or phosphorus levels are significantly altered.

Serum Calcium Determination:

In one method of practicing the present invention, the procedure for the determination of calcium is based on the interaction of the chromogenic agent o-cresolphthalein complexone (Sigma Diagnostics Calcium Agent, Sigma Chemical Co., St. Louis, MO) which complexes with calcium cation in an alkaline medium to form a purple colored complex which has an absorbance maximum at 575 nm. The intensity of the color measured at 575 nm is directly proportional to the calcium concentration in the given sample. The o-cresolphthalein complexone contains 8-hydroxyquinoline which prevents interference from magnesium ions. *Robertson, W.G.*, et al., "Calcium Measurements In Serum and Plasma - Total and Inonized," *Crit. Rev. Clin. Lab. Sci.*, Vol. 11, pg. 271, (1979); Sigma Diagnostics Product Insert: Calcium, Procedure No. 587, Reissued May 1989. The calcium level in serum is in the range of about 9.2 to 11.0 mg/dl which is composed of 3 distinct fractions.

Specimen Collection:

In one method of practicing the present invention, serum or heparinized plasma are suitable for the test. Anticoagulants other than heparin should not be used.

Procedure:

Sigma Diagnostics Calcium Reagent is used with Abbott Spectrum High Performance Diagnostic System for the quantitation of calcium in serum. It is generally considered good laboratory practice to run a calibration, linearity, and a quality control prior to the determination of patient samples. Calcium levels up to 16 mgs % can be measured by this method.

Serum Phosphorus Determination:

In one method of practicing the present invention, the procedure for the determination of serum phosphorus is based on the interaction of inorganic phosphorus with ammonium molybdate in the presence of sulfuric acid. *Barnhill S., et al.*, "Osteoporosis: A Possible Autoimmune Etiology," *Ann. of Clin. Lab. Sci.*, Vol. 17, pg. 255, (1987). This reaction produces unreduced phosphomolybdate complex. The absorbance of this complex at 340 nm is directly proportional to the inorganic phosphorus present in the given sample. (Sigma Diagnostics Product Insert: Phosphorus, Inorganic, Procedure No. 360-UV. Previous Revision December 1985. Reissued July (1988)). Most of the phosphorus in extracellular fluid is inorganic and in the range of 2.4 to 4.7 mg/dl.

Specimen Collection:

Serum or heparinized plasma are preferred for the test. Anticoagulants other than heparin should not be used.

Procedure:

Sigma Chemical Company's phosphorus inorganic reagent can be used with the Abbott Spectrum High Performance Diagnostic System for qualification of the inorganic phosphorus in serum. It is customary to perform calibration, linearity, and quality control studies prior to the determination of test samples. Phosphorus levels up to 12 mgs % can be measured by this procedure.

Total Alkaline Phosphatase:

The demonstration that bone is rich in alkaline phosphatase (ALP) and that normal serum contains the same or a similar enzyme led to the study of serum ALP levels in patients with disease of bone. See, *Courpron P.*, "Bone Tissue Mechanisms Underlying Osteoporosis," *Orthop. Clin. North Am.*, Vol. 12, page 513, (1981). This is especially true in osteitis deformans, hyperparathyroidism and bone neoplasm. ALP is also elevated in hepatic disease; however, this can be distinguished by other corroborative laboratory procedures and clinical features. In some situations, as in osteoporosis, ALP *per se* may not be above the reference range; however, the isoenzyme of ALP is increased. It is well understood that total serum ALP in normal subjects consists of isoenzymes contributed from liver, bone, renal, pulmonary, placental and intestinal sources, among others. The isoenzymes and isoforms of various tissue origins can be further separated and visualized by an Isoelectric Focusing Technique. Estimation of total serum ALP by isoelectric focusing techniques offers great potential in the investigation of metabolic bone diseases.

Serum Alkaline Phosphatase Determination:

In one method of practicing the present invention, serum ALP activity can be measured using various phosphate esters as substrates. Sigma Chemical Company's alkaline phosphatase reagent measures serum ALP activity by a kinetic method. The reagent for the test contains p-nitrophenyl phosphate, carbonate buffer, magnesium ions and mannitol. Mannitol present in the reagent acts as a phosphate acceptor during the enzyme reaction. *McComb R.B., et al.*, Alkaline Phosphatase. Plenum Press, New York, (1979); *Gundbery, M.*, Alkaline Phosphatase and Osteocalcin. Primer on Metabolic Bone Diseases and Disorders of Mineral

Metabolism 1st Edition, Published by Am. Soc. For Bone and Mineral Res. Editor-Murry J. Favus, pgs. 74-76, (1990); Sigma Diagnostics Product Insert: Alkaline Phosphatase (ALP), Procedure No. 245, Reissued March 1989; *Kaplan T.A.,et al.*, Clinical Chemistry. St. Louis, e.v. Mosby Company, (1987).

Specimen Collection:

Preferably, serum or heparinized plasma is used for the total alkaline phosphatase and alkaline phosphatase isoenzyme determination tests. EDTA, oxalate, citrate and fluoride are inhibitors of ALP and are not suitable anticoagulants.

Procedure:

Alkaline phosphatase (ALP) diagnostic reagent manufactured by Sigma Chemical Company is used with the Abbott Spectrum High Performance Diagnostic System for the quantitation of inorganic phosphorus in the serum. Serum ALP hydrolyzes p-nitrophenyl phosphate to p-nitrophenol and inorganic phosphate. The hydrolysis occurs at alkaline pH and the absorbance is directly proportional to the ALP activity of the serum sample. As is the usual practice, calibration, linearity and quality control assays should be performed prior to the determination of test samples.

ALP levels up to 1200 U/L can be measured by this procedure. Normal ranges are:

Infants	50-165	(U/L)
Adult	20-70 (U/L)	
Child	20-150	(U/L)
60 years	30-75 (U/L)	

Alkaline Phosphatase Isoenzyme:

Alkaline phosphatase isoenzyme levels have been primarily used to aid in the differential diagnosis of liver and bone disorders and also to indicate placental growth patterns. Isoelectric focusing of alkaline phosphatase isoenzyme on a precast gel gives 12 discernible bands of different organ and tissue origin. Of these 12 Bands, Band 10, appearing at 4.73 isoelectric point, is of placental origin. It is released by placental

syncytiotrophoblast and/or macrophage, and is T-lymphocyte mediated. In the alternative, samples for ALP isoenzyme can be analyzed by electrophoresis methods which identify mainly liver, bone, and intestinal isoenzymes. The Ciba Corning Alkaline Phosphatase Isoenzyme (ALP) System may be used for the qualitative and quantitative determination of ALP isoenzymes in human serum by electrophoresis.

The liver isoenzyme is the most frequently encountered alkaline phosphatase isoenzyme in pathologically increased serum alkaline phosphatase activity. Increased liver isoenzyme is encountered in a variety of liver and hepatobiliary diseases. These include hepatic cirrhosis, primary biliary cirrhosis, congestive cirrhosis, and hepatic carcinoma. Elevation of the liver isoenzyme is also a sensitive indicator of cholestasis and liver infiltration.

Another of the alkaline phosphatase isoenzymes that can be used in practicing the present invention is lymphocyte-derived alkaline phosphatase The preferred method of measuring this isoenzyme is by isoelectric focusing electrophoresis. A commercially available isoelectric focusing apparatus that is capable of measuring alkaline phosphatase isoenzymes is made by IsoLab, Inc. (Resolve®-ALP, IsoLab, Inc., Akron, Ohio). On the Resolve®-ALP isoelectric focusing apparatus, the alkaline phosphatase isoenzyme that is used in the present invention resolves as band 10 in the electrophoretic pattern. In the preferred bone density algorithm defined hereinbelow, the value for the band 10 alkaline phosphatase isoenzyme was assigned 0 if the band were missing or very weak, and 1 if the band were present. The electrophoresis gels can also be scanned with a densitometer and more quantitative values can be assigned to the alkaline phosphatase isoenzyme concentration. As used herein, the band 10 alkaline phosphatase isoenzyme shall be designated lymphocyte-derived alkaline phosphatase isoenzyme, or I-Alkp.

It is to be understood that other methods of measuring I-Alkp could be used. These methods include, but are not limited to, enzyme-linked immunoassay techniques (ELISA), radioimmunoassay techniques, affinity columns, and isoelectric focusing columns. It is important to note that measurement of band 10 on the Resolve®-ALP apparatus does not correlate at all with osteopenia in general. There are many other abnormal conditions

which result in a higher than normal band 10 lymphocyte-derived alkaline phosphatase isoenzyme (see *Barnhill, et al.* and *Griffith, et al.*, supra.).

A number of pathological conditions can lead to an elevated level of bone alkaline phosphatase isoenzyme. The highest levels of bone isoenzymes are usually found in Pagets disease. Increased levels are also encountered in rickets, bone cancer, osteomacacia and celiac sprue. Renal disorders can also result in increased levels. These include renal failure, primary hyperparathyroidism, secondary hyperparathyroidism induced by long term hemodialysis and malabsorption.

Increased levels of intestinal alkaline phosphatase are encountered in a variety of diseases of the digestive tract. These include intestinal infection and ulcerative lesions of the stomach, duodenum, small intestine and colon.

Procedure:

In the preferred method of practicing the present invention, the alkaline phosphatase isoenzymes are separated by electrophoresis in a buffered agarose system. After electrophoresis the isoenzymes are detected by incubating the gel with a fluorescent compound such as 4-methylumbelliferyl phosphate. The fluorescence formed during the interaction is quantitated using a Ciba-corning 710 densitometer at 385 nm. Total alkaline phosphatase activity of the patient is required to interpret the liver, bone and intestinal ALP isoenzymes.

Estrogen and Progesterone:

The estrogens are steroids that have a ring containing three unsaturated double bonds. The ovary, as well as the testes and adrenal gland, has the capacity to synthesize estrogens from androgens, androstenedione and testosterone. During the follicular phase of the menstrual cycle, ovarian secretion represents only one third of total estrogen production. In contrast to estradiol, which is secreted almost entirely by the ovary, most estrone is derived from peripheral conversion of androstenedione and from estradiol metabolism. During menopause, estradiol concentrations steadily decrease to approximately 15 percent of premenopausal levels. In healthy postmenopausal women, the ovaries do not secrete significant quantities of estrogens, and virtually all estrogen

produced is from peripheral conversion of androstenedione made by the adrenal. Estradiol is present in the serum as follows:

Follicular Phase	
Early	30-100 ng/L
Late	100-400 ng/L
Luteal Phase	50-150 ng/L
Postmenapausal	<20 ng/L

In menstruating females, progesterone is secreted mainly by the corpus luteum of the ovary. It is partially responsible for cyclic changes in the endometrium that are necessary for attachment and growth of an embryo. Progesterone levels are low prior to the mid-cycle gonadotropin surge. Shortly after the gonadotropin surge, they begin to rise rapidly, reaching peak levels during the middle of the luteal phase. Thereafter, a progressive fall occurs with barely detectable progesterone levels prior to menses. Although progesterone in large amounts produces a negative feedback on gonadotropin secretion, it is not the major component in the negative feedback system of ovarian steroids. Function of the corpus luteum can be assessed by measuring serum progesterone concentration. Progesterone is present in the serum as follows:

Follicular Phase	0.1 - 1.5 ng/L
Luteal Phase	2.5 - 28.1 ng/L
Mid-Luteal Phase	5.7 - 28.1 ng
Over 60 Years	0.0 - 0.2 ng/L

Procedure:

In a preferred method of practicing the present invention, progesterone measurements can be obtained by a radioimmuno-assay method using antibody coated tubes (Diagnostic Products, Los Angles, California). Estradiol measurements can be performed by a Microparticle Enzyme Immunoassay (IMX), available from Abbott Diagnostics, Abbott Park, IL.

Bone Mineral Density (BMD) Measurements

The conventional methods of diagnosing osteopenia which may be used when practicing the present invention are outlined below:

Dual-Photon absorptometry (DPA) is widely used to assess bone mineral content and bone mineral density. *Johnston Conrad C, et al.*, "Clinical Use of Bone Densitometry", *New Eng. J. of Med.*, Vol. 324, pgs. 1105-1109, (1991). DPA uses transmission scanning with photons from a radioisotope source, such as 153 Gd, that emits two energy peaks, thus allowing bone density to be measured independent of soft tissue. DPA measurements are performed on lumbar spine (L1-L4) and femur (femoral neck, Ward's Triangle and trochanter) and the average determined separately. The overall average of both hip and spine can also be determined. BMD can be measured by DPA using ~ Gadolinium as the source (Lunar DP3, by Lunar Radiation Corporation, Madison, Wisconsin).

The present method employs transmission scanning using 44 and 100 KeV photon energies from a one Ci^{153}Gd source to allow computation of the mineral content of bone independent of soft tissue thickness. Bone mineral density, expressed in g/cm^2, is derived by dividing bone mineral content (BMC) by the projected area of the scanned bone. *Peppler W.W., et al.*, "Total Body Bone Mineral and Lean Body Mass by Dual-Photon Absorptometry: 1. Theory and Measurement Procedure," *CALCIP, Issue Int.*, Vol. 33, pg. 353, (1981); *Shipp C., et al.*, "Precision of Dual-Photon Absorptometry," *CALCIP, Issue Int.*, Vol. 42, pgs. 287-292, (1988). During spine scans, the detector moves in a rectilinear pattern at a rate of 5mm/sec and with scan lines 4.5 mm apart. The BMC and BMD are calculated in lumbar vertebrae 1 through 4 (including intervertebral discs) with a software version supplied by Lunar Radiation Corporation of Madison, Wisconsin.

During femur measurements, the scanner moves at a rate of 2.5 mm/sec and a step distance of 2.5 mm. The BMC and BMD of the neck, Ward's Triangle and trochanteric regions of the proximal femur are calculated using a femur software version supplied by Lunar Radiation Corporation. The femoral neck region of interest (ROI) is that band about 1.5 cm wide across the neck of the bone perpendicular to the neutral axis with the lowest density. Ward's Triangle is defined as a square ROI (about 1.5 x 1.5 cm) with the lowest density within the proximal femur region.

Ward's Triangle is predominantly trabecular bone and contains the least amount of bone mineral within the neck region. Carter D.R., et al., "Relationship Between Loading History and Femoral Cancellous Bone Architecture," *J. Biomechanics*, Vol. 22, pgs. 231-244, (1989). In the proximal femur, the ROI is usually the area 1.5 cm wide across the entire femoral neck. Additional regions are defined by the software in the lower density Ward's Triangle region, and in the region of the greater trochanter. Bone loss in the proximal femur begins in the Ward's Triangle region and proceeds outward from there. (*Brown D., et al.*, "Mechanical Property Distributions in the Cancellous Bone of the Human Proximal Femur," *Act. Orthop. Scand.*, Vol. 51, pgs. 429-437, (1990). This makes the region an early indicator of bone loss, but the higher variance in measuring it, compared to the neck region, makes the latter zone, a better discriminator. However, Ward's Triangle is the lowest density area at the point where the neck and greater trochanter meet, a primary hip fracture site. This operational ROI may not correspond exactly to the anatomic Ward's Triangle region but does provide a repeatable measurement. The width of the neck ROI and the size of the Ward's Triangle ROI are actually proportional to the measured size of the femoral neck. It has been shown that the density of the Ward's Triangle area is substantially reduced in hip fracture patients compared with age matched controls. (*Vose G. P., et al.*, "Femoral Neck Fracturing its Relationship to Radiographic Bone Density," *J. Gerontol.*, Vol. 20, pgs. 300-305, (1965). DPA measurements have a precision of 1-3% and the scan can be completed in about 20 minutes. Bone density of vertebrae correlates well with risk for vertebral fracture, and bone density of areas in the proximal femur correlates well with risk for hip fracture. DPA has a low radiation dose (<10 mrem to skin, and 2 mrem to marrow). DPA has approximate sensitivity of spine and hip of 50% and 53%, respectively, at 95% specificity (i.e. % of fracture cases below 5th percentile). Moreover, DPA is not subject to systematic errors introduced by variable osteoid and variable marrow.

Bone density can also be measured by radiographic absorptometry. Radiographic absorptometry is a method of measuring bone density which is well known to those of ordinary skill in the art. Other methods of measuring bone density include quantitative computed tomography and direct measurement of bone density.

Direct measurement of bone density can be obtained by measuring the bone density in cadavers. Thus, another method of determining the correlation between the blood concentrations of certain predetermined blood constituents and bone density is to measure the bone density of a set of cadavers from which blood concentrations of predetermined blood constituents are known. The multiple linear regression analysis can then be performed and a bone density coefficient relationship can easily be determined.

In correlating the bone density measurements to concentration of blood constituents, blood concentrations of calcium, phosphate, total alkaline phosphatase, an alkaline phosphatase isoenzyme estradiol, and progesterone are measured. Liver, bone and intestinal isoenzymes can be used. Band 10 alkaline phosphatase isoenzyme. A mathematical relationship between the concentrations of blood constituents and bone density, as measured by radiographic absorptometry or other standard method of measuring bone density, is determined by performing a multiple regression analysis with the following model:

Bone density coefficient $= b_0 + b_1Ca + b_2P + b_3E2 + b_4Pg + b_5Alkp + b_6I\text{-}Alkp + b_7(CaCa) + b_8(PP) + b_9(E2E2) + b_{10}(Pg + Pg) + b_{11}(AlkpAlkp) + b_{12}(CaP) + b_{13}(CaE2) + b_{14}(CaPg) + b_{15}(CaAlkp) + b_{16}(CaI\text{-}Alkp) + b_{17}(PE2) + b_{18}(PPg) + b_{19}(PAlkp) + b_{20}(PI\text{-}Alkp) + b_{21}(E2Pg) + b_{22}(E2Alkp) + b_{23}(E2I\text{-}Alkp) + b_{24}(PgAlkp) + b_{25}(PgI\text{-}Alkp) + b_{26}(AlkpI\text{-}Alkp)$

where:

Ca = Serum concentration of calcium

P = Serum concentration of phosphorus

E2 = Serum concentration of estradiol

PG = Serum concentration of progesterone

Alkp = Serum concentration of total alkaline phosphatase

I-Alkp = Serum concentration of alkaline phosphatase isoenzyme.

Calculation of the mathematical model utilized the Systat® statistical package (Systat: Inc., Evanston, IL). The multiple linear regression analysis is an iterative process which calculates the correct coefficients so that the result of the algorithm using the blood concentrations correlates to a

high degree with the result of the bone density measurement by radiographic absorptometry.

For diagnosing osteopenia, the general form of the preferred algorithm that is used in the present invention is as follows:

Bone density coefficient = $b_0 + b_1$Ca + b_2P + b_3E2 + b_4Pg + b_5Alkp + b_6I-Alkp + b_7(CaCa) + b_8(PP) + b_9(E2E2) + b_{10}(Pg + Pg) + b_{11}(AlkpAlkp) + b_{12}(CaP) + b_{13}(CaE2) + b_{14}(CaPg) + b_{15}(CaAlkp) + b_{16}(CaI-Alkp) + b_{17}(PE2) + b_{18}(PPg) + b_{19}(PAlkp) + b_{20}(PI-Alkp) + b_{21}(E2Pg) + b_{22}(E2Alkp) + b_{23}(E2I-Alkp) + b_{24}(PgAlkp) + b_{25}(PgI-Alkp) + b_{26}(AlkpI-Alkp)

where:

b_0 is a constant

Ca = Serum concentration of calcium

P = Serum concentration of phosphorus

E2 = Serum concentration of estradiol

PG = Serum concentration of progesterone

Alkp = Serum concentration of total alkaline phosphatase

I-Alkp = Serum concentration of alkaline phosphatase isoenzyme.

It is to be understood that concentration of I-Alkp can be replaced with serum concentration of liver, bone or intestinal derived alkaline phosphatase isoenzymes.

In practicing the present invention, the values obtained from the patient for the six blood constituent concentrations are inserted into the algorithm as indicated. The mathematical manipulation is performed. The resulting number is called a bone density coefficient and is then placed in the severity scale. This results in a bone density probability quotient which correlates to a high degree to bone density as measured by radiographic methods.

As seen in the algorithm that is considered part of the present invention, there are a number of coefficients which are a part of the algorithm. It is to be understood that these coefficients can change if the bone density coefficient is correlated to a different method of determining bone density. Thus, if bone density is measured using dual photon absorptometry, and one wanted to correlate the bone density coefficient to

the results of the dual photon absorptometry, the overall relationship of the tests in the algorithm would be the same or similar as disclosed herein but the coefficients could be different.

In its preferred embodiment, the bone density probability quotient is assigned to one of the following diagnostic categories:

Normal to mild osteopenia
Moderate osteopenia
Severe osteopenia

In addition, the menopausal status may be determined based on the results of the estradiol and progesterone levels into one of the following diagnostic categories:

Probable Pre-menopausal
Probable Peri-menopausal
Probable Post-menopausal

Thus, according to the present invention, using blood concentrations of certain blood constituents, one can not only diagnose bone density but can also obtain an indication of the underlying cause of the osteopenia.

This invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. To the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

Example 8

In the following example, the bone density coefficient algorithm was correlated with bone density as measured by radiographic absorptometry. The coefficients were calculated by correlating the blood concentration of the indicated blood constituents in 27 patients to the results of bone density in those patients as measured by radiographic absorptometry. The bone density algorithm which results is as follows:

$$0 + (-8.955)(Ca) + (79.370)(P) + (34.076)(E2) + (-9.216)(Pg) + (-0.600)(Alkp) + (-57.855)(I\text{-}Alkp) + (0.926)(CaCa) + (-2.735)(PP) + (-0.272)(E2E2) + (-0.064)(PgPg) + (0.004)(AlkpAlkp) + (-4.029)(CaP) + (-3.156)(CaE2) + (1.172)(CaPg) + (-0.031)(CaAlkp) + (8.238)(CaI\text{-}Alkp) + (1.906)(PE2) + (-0.138)(PPg) + (-0.018)(PAlkp) + (-6.710)(PI\text{-}Alkp) + (0.193)(E2Pg) + (-0.048)(E2Alkp) + (-1.825)(E2I\text{-}Alkp)$$

wherein:

Ca = Serum concentration of calcium

P = Serum concentration of phosphorus

E2 = Serum concentration of estradiol

PG = Serum concentration of progesterone

Alkp = Serum concentration of total alkaline phosphatase

I-Alkp = Serum concentration of serum lymphycyte-derived alkaline phosphatase isoenzyme.

The bone density coefficient that is obtained from the bone density algorithm is assigned to one of three categories:

Group 1	normal to mild osteopenia
Group II	moderate osteopenia
Group III	severe osteopenia

Group III ≤ approximately 78.5 < Group II ≤ approximately 100 < Group I

It has been determined that the bone density coefficient calculated according to the present invention places patients in the same groups as radiographic absorptometry measurements for bone density. Thus, the present invention provides a safe, economical and accurate method for diagnosing osteopenia.

Bone density quotients derived practicing the present invention using the six biochemical serum constituents were found to strongly correlate with bone density measurements from radiographic absorptometry. It is to be understood that the preferred embodiment utilizes all six of the biochemical serum constituents listed hereinabove. However, if any five of the serum constituents are utilized and then included in the bone density algorithm, a

bone density quotient is obtained which does not correlate as well as when six tests are used.

It is contemplated as part of the present invention that any five of the biochemical serum constituents can be used to determine the osteopenic state of a patient. For example, if serum calcium is removed from the bone density algorithm, then the algorithm predictive value for moderate or severe osteopenia is approximately 66% although the predictive value for normal to mild osteopenia is 100%. If progesterone is removed from the bone density algorithm, then the algorithm predictive value for moderate or severe osteopenia of approximately 75% and a predictive value for normal to mild osteopenia of approximately 94%. If alkaline phosphatase is removed from the bone density algorithm, then the algorithm predictive value for moderate or severe osteopenia of approximately 83% and a predictive value for normal to mild osteopenia of approximately 85%. If estradiol is removed from the bone density algorithm, then the algorithm predictive value for moderate or severe osteopenia of approximately 67% and a predictive value for normal to mild osteopenia of approximately 85%. However, it has been determined that when all six tests are included in the bone density algorithm, the predictive value for moderate and severe osteopenia is 100% and the predictive value for normal to mild osteopenia is 100%. All of the predictive values may vary slightly with different patient populations.

Example 9

The following serum constituents are measured in a 37 year old woman:

Calcium	9.5 mg/dL
Phosphate	5.0 mg/dL
Estradiol	2 pg/mL
Progesterone	0.2 ng/mL
Alkaline Phosphatase	80 U/L
Alkaline phosphatase isoenzyme	0 (negative)

The above blood chemistry values were used to calculate a bone density coefficient using the following algorithm:

0 + (-8.955)(Ca) + (79.370)(P) + (34.076)(E2) + (-9.216)(Pg) + (-0.600)(Alkp) + (-57.855)(I-Alkp) + (0.926)(CaCa) + (-2.735)(PP) + (-0.272)(E2E2) + (-0.064)(PgPg) + (0.004)(AlkpAlkp) + (-4.029)(CaP) + (-3.156)(CaE2) + (1.172)(CaPg) + (-0.031)(CaAlkp) + (8.238)(CaI-Alkp) + (1.906)(PE2) + (-0.138)(PPg) + (-0.018)(PAlkp) + (-6.710)(PI-Alkp) + (0.193)(E2Pg) + (-0.048)(E2Alkp) + (-1.825)(E2I-Alkp)

where:

Ca = Serum concentration of calcium

P = Serum concentration of phosphorus

E2 = Serum concentration of estradiol

PG = Serum concentration of progesterone

Alkp = Serum concentration of total alkaline phosphatase

I-Alkp = Serum concentration of serum lymphycyte-derived alkaline phosphatase isoenzyme.

A bone density coefficient of 101.2 is calculated for this patient. This coefficient falls within Group I in the following severity scale:

Group III ≤ 78.5 < Group II ≤ 100 < Group I

Group I	normal to mild osteopenia
Group II	moderate osteopenia
Group III	severe osteopenia

This patient has normal to mild osteopenia. When the patient's bone density is measured by radiographic absorptometry, she is found to have normal bone density.

Example 10

The following serum constituents are measured in a 47 year old woman:

Calcium	9.5 mg/dL
Phosphate	5.0 mg/dL
Estradiol	2 pg/mL
Progesterone	0.2 ng/ml
Alkaline Phosphatase	80 U/L
Alkaline phosphatase isoenzyme	1 (positive)

The values are inserted into the bone density algorithm recited in Example I and a bone density coefficient of 84.4 is calculated. This falls in Group II, indicating a moderate osteopenia. When the patient's bone density was measured by radiographic absorptometry, she was found to be moderately osteopenic.

Example 11

The following serum constituents are measured in a 47 year old woman who has been diagnosed with breast cancer:

Calcium	9.0 mg/dL
Phosphate	4.0 mg/dL
Estradiol	0 pg/mL
Progesterone	0 ng/mL
Alkaline Phosphatase	60 U/L
Alkaline phosphatase isoenzyme	1 (positive)

The values are inserted into the bone density algorithm recited in Example I and a bone density coefficient of 69.9 is calculated. This falls in Group III indicating a severe osteopenia insured by another method.

Example 12

The following example shows the correlation between the method of the present invention and Dual Photon Absorptometry (DPA) as a tool for diagnosing and determining the severity of osteoporosis in a particular individual. 200 female subjects representing a cross section of ages and menopausal status were evaluated. These specific age groups were represented as follows:

Age	Number of Patients
25-35	26
36-45	25
46-55	33
56-65	30
66-75	34
76-85	25
85 and over	26

Each subject completed an Osteoporosis Data Questionnaire Form, which included detailed information on personal history, family history, gynecologic history, medical history, surgical history, and drug history, with special references to hormone treatment.

Three tubes of blood were collected from each subject via venipuncture. One tube was sent to Barnhill-MetPath Laboratories in Savannah, Georgia for analysis of serum calcium, phosphorous, and total alkaline phosphatase. The second tube was sent to Horus Therapeutics, Inc. in Savannah, Georgia for determination of lymphocyte alkaline phosphatase utilizing the Isoelectric Focusing Method. The third tube was sent to MetPath Laboratories in Teterboro, New Jersey for evaluation of estradiol and progesterone by immunoassay. Data from all three sources were then subjected to statistical analysis. At the time of the analysis of data, each subject's chart was critically reviewed for relevant clinical information.

The Bone Mineral Density (BMD) of each subject was also measured by Dual-Photon Absorptometry using ^{153} Gadolinium as a source (using Lunar DP3, Lunar Radiation Corporation, Madison, Wisconsin). DPA measurements were performed on the Lumbar Spine (L1-L4) and the hip, specifically the femoral neck, Ward's Triangle, and the trochanteric regions. BMD was measured in gm/cm^2 for each region noted, and the fracture risk was determined.

The BMD measurements were then age matched, and adjusted for sex, age, ethnic group, and weight. From the individual values obtained, average BMD for hip and spine was calculated. The overall average BMD for both hip and spine was also computed.

A mathematical algorithm correlating the results using the present invention with DPA was determined with the resultant data is shown in Figure 11.

Figure 12 illustrates the discrepancies between the DPA measurements and the measurements obtained using the method of the present invention for individual subjects. The amount of the discrepancy (the residual) for any patient is the vertical distance between the points for that patient on the DPA and the present invention lines.

Figure 13 illustrates the appropriateness of using the present invention as a prediction of DPA measurements. Note the absence of any tendency for points to be consistently above or consistently below the diagonal straight line, as greater DPA measurements are considered. Additionally, there is an absence of any tendency for points along any vertical line to be more widespread than are points along any other vertical line. Finally, note that the generally elongated shape formed by the points is consistent with a strong linear correlation between DPA and the present invention.

Figure 14 demonstrates the normality of values predicted by the present invention (the horizontal scale). The vertical scale shows the of the normalized scale (z) scores values determined according to the present invention as would be expected from the assumption of the present invention values forming a normally distributed collection of numbers.

The closer the dots are to the straight line, the greater is the consistency between what is observed about the present invention values and what is expected about them from the assumption of their forming a normally distributed collection of numbers.

Figure 15 demonstrates the normality of residuals (the difference between the observed DPA measurement and the value predicated by present invention), as evidenced by the arrangements of points along the diagonal straight line. The horizontal scale shows values of residuals The vertical scale shows the residuals' normalized scale (z) scores as would be expected from the assumption that the residuals form a normally distributed collection of numbers.

The closer the dots are to the straight line, the greater is the consistency between what is observed about the residuals and what is

expected about them from the assumption of their forming a normally distributed collection of numbers.

Figure 16 demonstrates that the variance of residuals stays the same regardless of the value calculated according to the present invention involved in the residuals' computation. This homogeneity of variance appears in the uniform density of the dots in a rectangle formed by residuals between -0.17 and +0.17 and by the present invention between +0.4 and +1.0. Moreover, there is no discernable tendency for points along any vertical line to be more widespread than are points along any other vertical line.

Figure 17 demonstrates the independence of residuals and the values predicted by the present invention. The horizontal scales shows the values predicted by the present invention. The vertical scale shows the residual's studentized scale (t) scores.

When there is no relationship between values predicted by the present invention and the residuals involving those predicted values (that is, when there is independence between residuals and predicted present invention values) the following criteria should be met:

1. There should be no discernable pattern (such as a straight or curved line) to the dots.
2. About half the dots should appear in the top half of the graph.

Figure 18 demonstrates that virtually all observed DPA measurements are satisfactorily predicted by the method of the present invention. The horizontal scale shows values predicted by the method of the present invention. The vertical scale shows Cook's distances between observed DPA measurements and the predictions of those measurements by the method of the present invention Thus, the closer the dots are to a height of Cook = 0, the more accurate is the method of the present invention. Because Cook's distances tend to form a collection of numbers fitting an F-statistical distribution, the two outline points at heights between 10 and 20 are not thought to be problematic in view of the large size of the sample.

Example 13

The following example describes the training of a neural network to prognose prostate cancer.

As shown in Fig. 19, a total of 52 samples were divided into 2 groups, a training set and a generalization testing set. The training set contained 40 samples (28 stable and 12 progressing) and the generalization testing set contained 12 samples (9 stable and 3 progressing).

The initial network architecture was selected based on the level of complexity of the classification task. A multi-layer feedforward network was used. Selection of the initial architecture involved the selection of the number of hidden layers and the number of neurons in each hidden layer. Several trial iterations were performed to determine and adequate configuration that showed good results on both the training sample set and the generalization test sample set. The present network had one hidden layer, having nine neurons, and two output neurons.

Initially, connection weights among the neurons were randomly set. The neural network had five input neurons, corresponding to five input variables significant for prostate cancer: TPS, PSA, PAP, CEA, and testosterone. The training data is shown in Fig. 19. During training, the five input variables for each patient were first linearly scaled into the continuos range between 0.0 and 1.0. The resultant five numbers were then presented as an input vector to the input neurons of the artificial neural network.

For each of the input vectors, the network generated an output based on the connection weights among the network neurons. The output can be a single value or a vector of numbers, depending on the number of output neurons used. The network used had two output neurons. The outputs of the two neurons were processed by the following mathematical equation to yield a single diagnostic index:

$$\text{Index} = \frac{(\text{ANN2}-\text{ANN1})}{2} + 0.5$$

Each neuron in the network participated in the output calculation by passing the sum of all inputs to the neuron through a non-linear s-shaped function (often a ligistic function) and sending the result to each and everyone of the

neurons in the following adjacent layer. The generated output or each output neuron was compared to the desired "target" output. A value of 0.1 corresponded to a diagnosis of stable and an output of 0.9 corresponded to a diagnosis of progressing. The difference was used to calculate an error term to guide the training algorithm, i.e., the back propagation algorithm, in the adjustment of network connection weights in an attempt to reduce the differences between network outputs and target values over the training sample set.

After training, the neural network correctly classified 100% of the samples.

When presented with the generalization test results, the trained neural network correctly identified 100% of the stable samples and 66% of the samples where the disease was progressing.

It should be understood, of course, that the foregoing relates only to preferred embodiments of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in the appended claims.

Claims

What is claimed is:

1. A method for diagnosing a disease in a human or animal comprising the steps of:

measuring the concentrations of a predetermined set of biomarkers known to be associated with the disease from a biological fluid from the human or animal;

scaling the digitized values of the biomarkers concentrations; and

introducing the scaled values to a trained neural network means, whereby the output values from the neural network means tend toward the upper value when the human or animal has the disease and the output values tend toward the lower value when the human or animal does not have the disease.

2. An apparatus for diagnosing a disease in a human or animal comprising:

means for digitizing the concentrations of a predetermined set of biomarkers known to be associated with the disease from a biological fluid from the human or animal;

a means for scaling the digitized values; and

a trained neural network means coupled to the digitizing and scaling means for generating network output values between an upper and lower value, whereby the output values tend toward the upper value when the human or animal has the disease and the output values tend toward the lower value when the human or animal does not have the disease.

3. A method for diagnosing a disease in a human or animal comprising the steps of:

measuring the concentrations of a predetermined set of biomarkers known to be associated with the disease from a biological fluid from the human or animal;

scaling the digitized values of the biomarkers concentrations;

introducing the scaled values to a first trained neural network means, whereby the output values from the first neural network means tend toward the upper value when the human or animal has the disease and the output values tend toward the lower value when the human or animal does not have the disease; and

introducing the output value from the first neural network and a second set of predetermined biomarkers to a second trained neural network means, whereby the output values from the second neural network means tend toward the upper value when the human or animal has the disease and the output values from the second neural network means tend toward the lower value when the human or animal does not have the disease.

4. The method of Claim 3 wherein the second set of predetermined biomarkers includes patient biographical information.

5. The method of Claim 3 wherein the second set of predetermined biomarkers are the same as the first set of predetermined biomarkers.

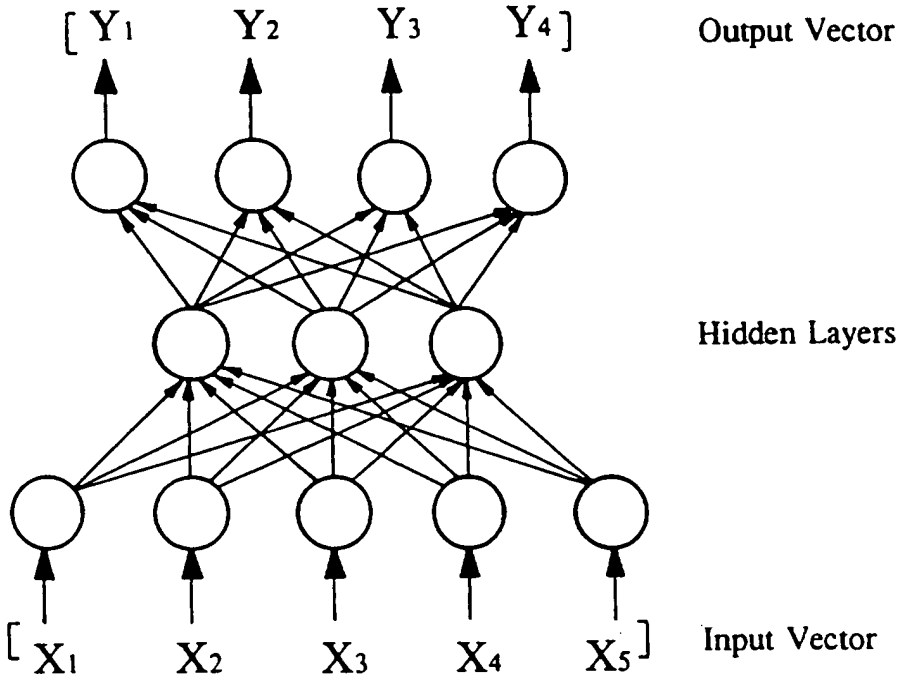
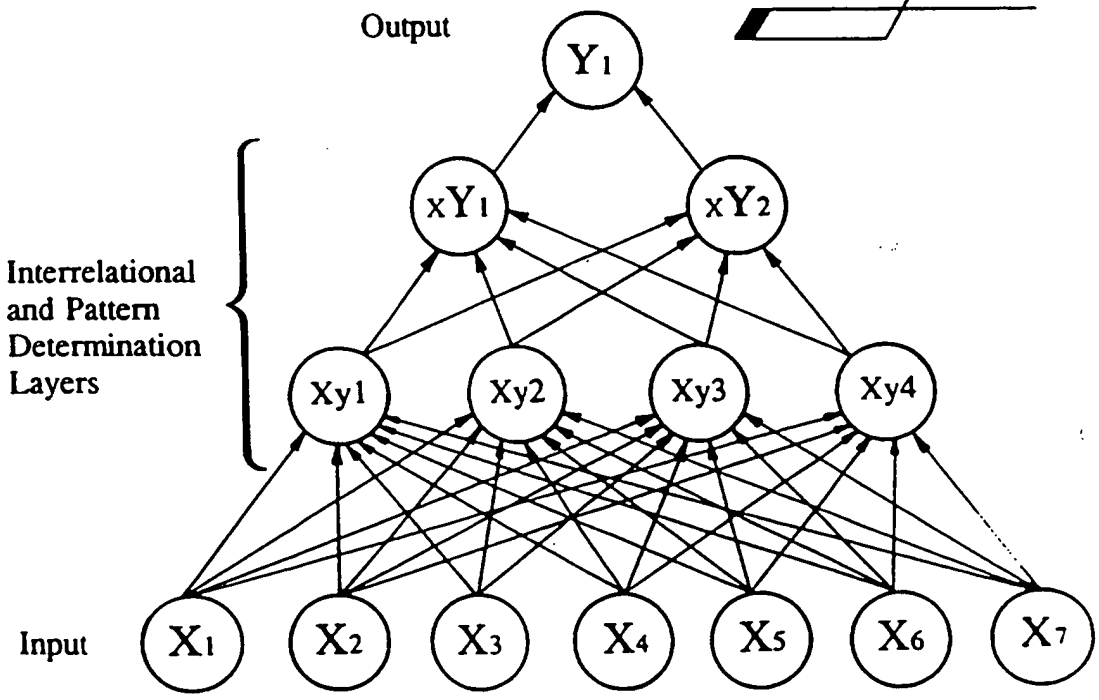


Fig_1

Fig_2



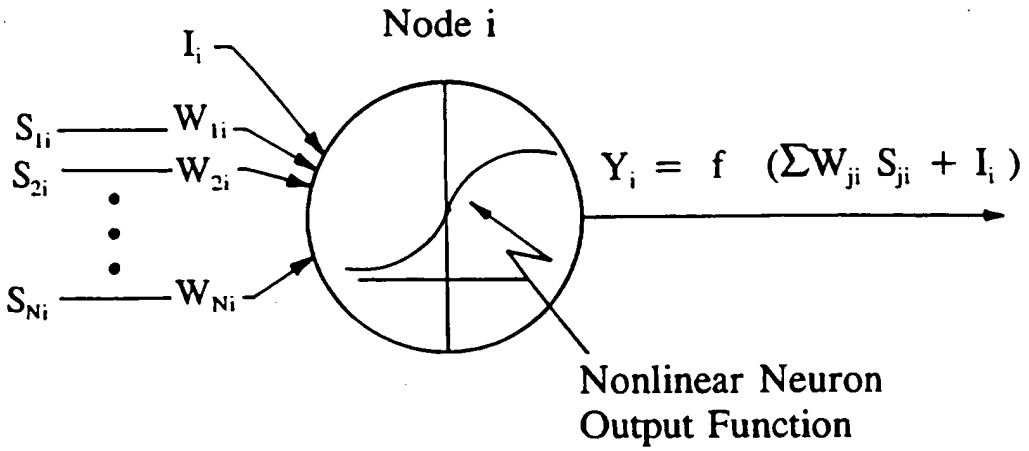


Fig_3

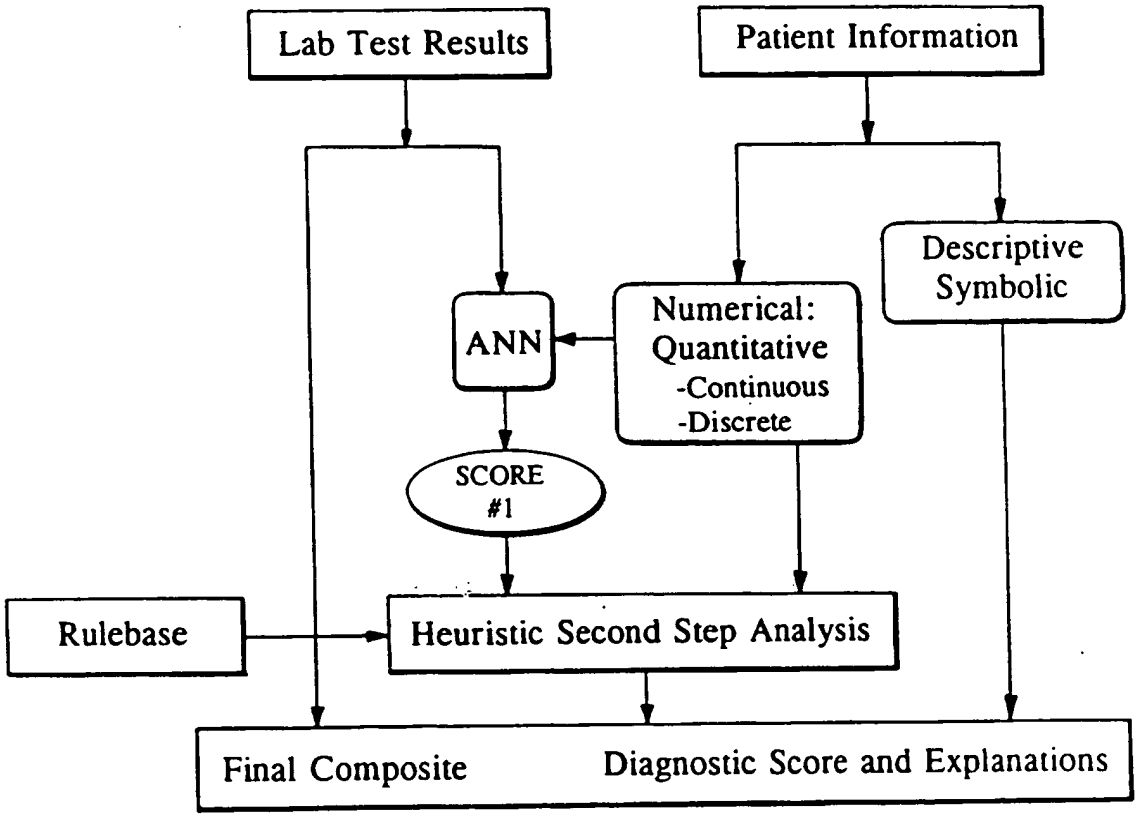


Fig_4

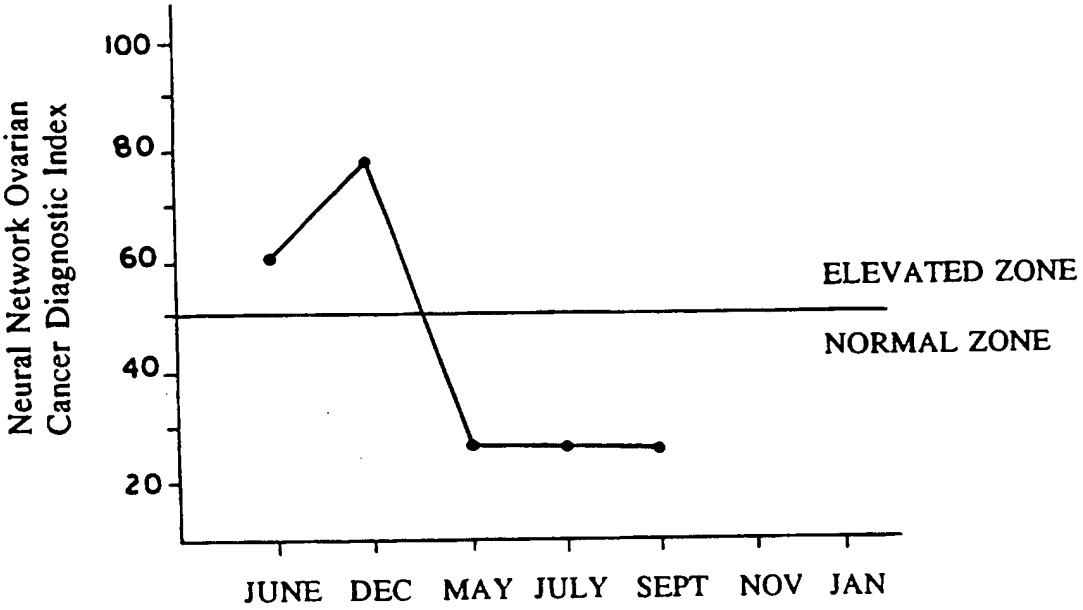


Fig_5

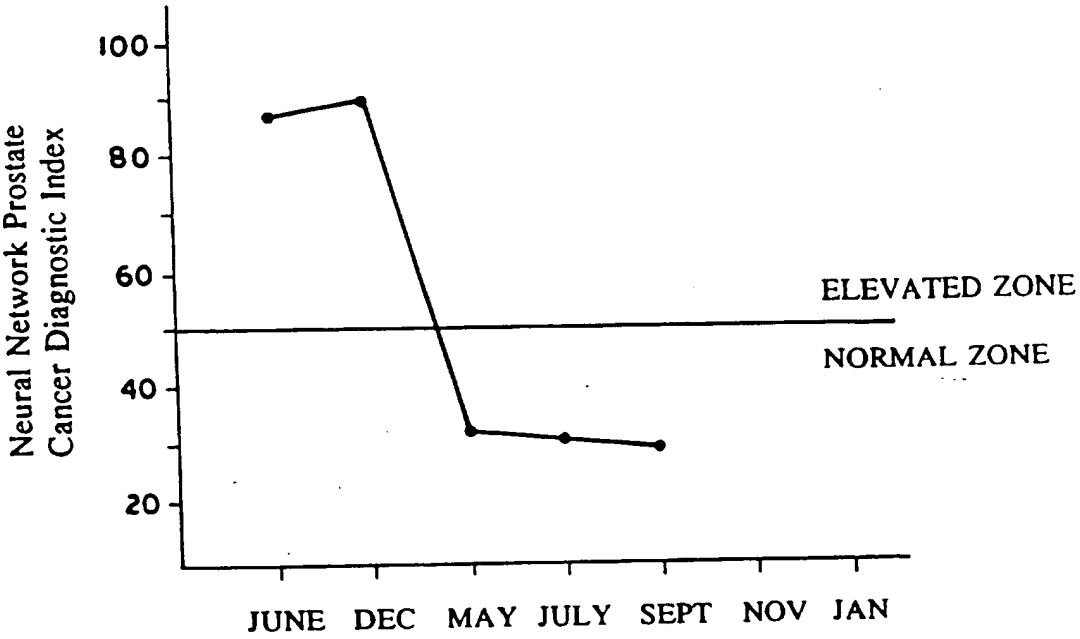


Fig_6

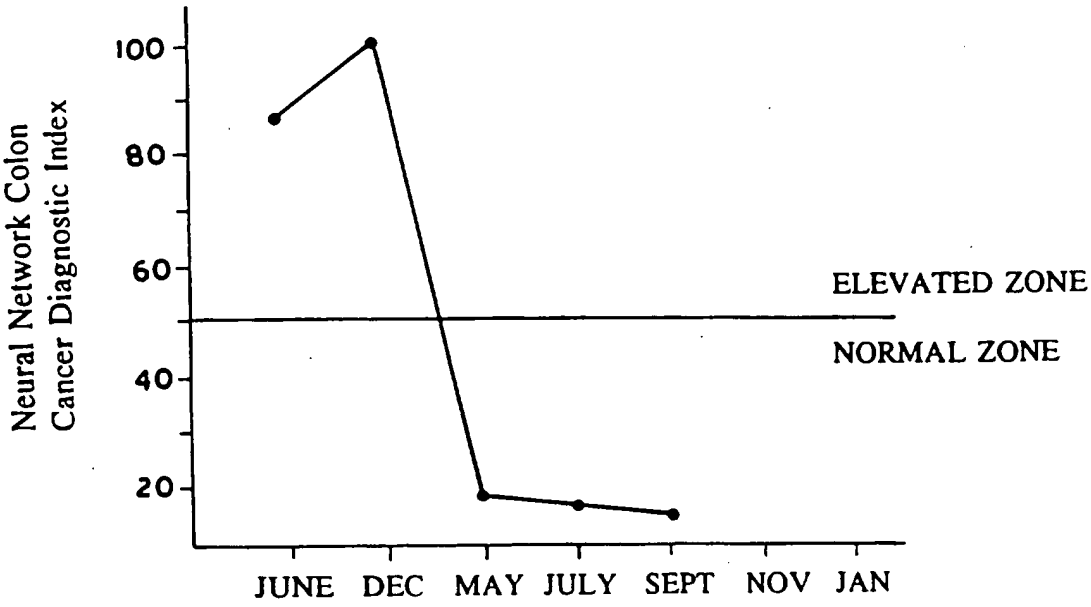


Fig_7

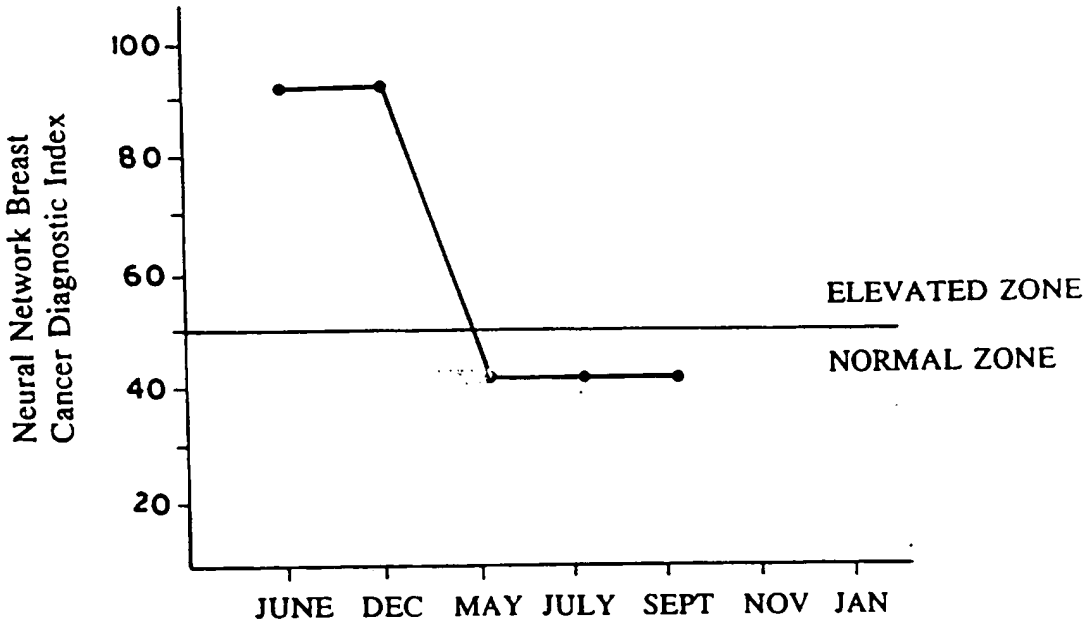


Fig_8

Training Data for Osteoporosis Neural Network Diagnostic System

PATIENT	AGE	CAL	PHOS	ETWO	PROG	TALP	TINTES	PLIVER	SCORE
1	61	9.7	3.6	15	0.2	82	0	67.317	0.645
2	55	9.2	3.9	83	7.9	31	3.7	49.032	0.747
3	33	9.4	3.6	12	0.1	39	2.8	62.821	0.819
4	43	8.5	4	4	0.3	39	2.6	50.513	0.841
5	58	9.5	3.5	40	0.1	75	0	78.267	0.660
6	48	9.6	3.8	145	7.5	49	0	59.388	0.679
7	56	8.6	3.8	43	0.1	48	0	60.833	0.698
8	36	9.4	4.1	106	6	77	0	54.156	0.819
9	32	8.5	3.6	18	0.7	52	0	51.538	0.810
10	33	9.4	3.6	65	2.4	63	4.6	70.794	0.815
11	74	9.2	4.2	13	0.2	70	0	62.571	0.619
12	41	8.8	4.1	43	0.7	70	13.1	48.000	0.890
13	28	9.1	3	84	0.3	76	7	52.763	0.802
14	44	9.1	3.3	176	0.6	82	10.3	62.805	1.011
15	62	9.1	3.4	90	0.2	32	0	40.625	0.693
16	36	9.6	3.8	59	0.1	39	0	66.154	0.822
17	60	8.9	3.4	129	0.1	64	0	78.125	0.668
18	42	9.3	3.8	35	0.8	43	0	39.070	0.814
19	35	9.4	3.7	58	0.2	39	0	55.128	0.828
20	47	9.1	5.2	42	1.4	50	7.6	60.200	0.762
21	46	9	4.5	95	7	52	10.4	60.192	0.886
22	37	9.3	3.4	231	0.2	60	0	84.667	0.779
23	30	9.1	4.2	5	0.2	61	11.3	52.459	0.837
24	45	9.2	3.6	63	2.3	44	5.4	64.318	0.766
25	42	9.2	3.5	139	0.4	44	0	72.045	0.773
26	61	9.8	4	22	0.3	70	0	49.571	0.669
27	64	8.8	4.1	17	0.2	73	5.7	65.068	0.646
28	79	10.2	3.9	13	0.1	67	0	53.731	0.602
29	33	8.5	2.9	20	0.4	39	5.2	47.949	0.791
30	41	9	2.5	120	0.8	56	0	55.893	0.770
31	50	9	3	79	0.7	49	0	48.367	0.721
32	46	9.5	4.1	22	0.3	58	4	46.034	0.729
33	51	9.2	3.2	162	0.3	58	0	50.172	0.716
34	45	9	3.1	44	2	42	0	65.714	0.733
35	45	9.3	3.8	123	12.8	66	18.3	54.848	0.965
36	32	9.3	3.8	40	0.6	40	4.3	39.000	0.849
37	51	8.9	3.6	71	2.8	70	7.1	55.429	0.713
38	29	9.2	4	117	16.5	30	0	57.000	0.894
39	31	9.3	4	93	0.3	41	0	25.610	0.885
40	69	10	4.2	15	0.3	110	36.4	45.364	0.615
41	29	9.2	3.9	12	0.1	147	18.1	54.694	0.770
42	67	9.3	4.7	3	0.2	65	2.9	48.000	0.663
43	42	8.8	3	57	2.5	55	3.9	31.091	0.827
44	52	9.6	3.8	118	0.6	53	3.8	63.774	0.733
45	51	10	4.4	52	0.3	62	8.6	39.516	0.745
46	46	8.8	3.6	73	3	45	0	26.444	0.788
47	46	8.9	4.4	14	0.4	64	0	62.031	0.783

Fig_9

PATIENT	AGE	CAL	PHOS	ETWO	PROG	TALP	TINTES	PLIVER	SCORE
48	45	9.1	4.5	206	0.2	84	19.6	45.357	0.843
49	81	9.3	4.2	4	0.3	66	7.4	64.848	0.594
50	32	9	2.5	25	1.4	56	0	68.929	0.763
51	52	9.5	3.8	11	0.1	49	0	66.735	0.714
52	47	8.9	4.7	15	0.2	64	7	66.563	0.744
53	60	9.2	3	18	0.2	101	23.8	39.604	0.622
54	47	9.5	4.5	8	0.1	72	8.2	66.389	0.702
55	46	9.5	3.5	34	10.6	67	9.8	54.925	0.767
56	57	10.1	3.9	38	0.4	120	9.6	73.083	0.649
57	69	9.5	3.7	10	0.1	91	0	68.132	0.612
58	62	8.7	3.5	13	0.2	65	0	52.615	0.650
59	73	8.9	4	15	0.2	102	11.1	60.294	0.594
60	51	8.9	3.5	74	4.4	51	0	69.608	0.726
61	51	9	3.7	12	0.4	109	0	56.330	0.667
62	63	9.5	4.7	9	0.3	51	0	51.961	0.692
63	77	9.3	3.2	17	0.2	106	10.7	51.226	0.567
64	63	9.2	4.1	17	0.2	51	7.9	31.569	0.675
65	73	9.6	1.1	19	0.2	69	0	75.217	0.576
66	83	9	3.6	2	0.1	57	0	81.404	0.581
67	59	8.8	3.7	24	0.1	85	6.9	59.294	0.648
68	60	8.9	4	110	0.3	40	0	66.500	0.706
69	76	9.4	3.5	21	0.1	54	5.8	62.593	0.604
70	59	9.2	4.6	15	0.1	60	0	51.500	0.699
71	63	9.2	3.4	121	0.1	48	0	140.833	0.667
72	63	9.4	3.2	65	0.1	64	8.3	62.188	0.648
73	76	8.9	3.8	8	0	91	11.8	58.132	0.587
74	46	8.6	3.5	54	7.4	73	10.1	46.301	0.993
75	57	9.1	3.7	195	0.1	61	11.4	55.410	0.695
76	41	9.1	3.4	67	3	57	0	68.772	0.733
77	61	9.9	4.3	20	0.1	61	8.6	57.213	0.679
78	25	9.1	3.3	122	0.6	62	0	66.935	0.821
79	51	9.2	3.1	124	7.7	79	11.1	26.835	0.704
80	57	8.9	3.9	15	0.3	84	0	72.500	0.662
81	76	8.8	3.1	12	0.2	88	0	86.023	0.574
82	64	9.3	3.4	131	0.1	57	4.5	55.965	0.662
83	61	9.4	3.5	22	0.5	86	9.4	60.581	0.637
84	42	9.3	3.4	150	14.9	78	0	54.744	0.699
85	28	8.9	3.5	177	9.6	46	0	34.348	0.867
86	26	9.4	4.1	139	0.2	52	6.9	52.500	0.878
87	73	9.3	3.5	0	0.2	72	11.5	52.361	0.604
88	37	9.3	3.1	55	2.2	77	6.4	56.623	0.756
89	72	9.2	4.3	4	0.2	111	6.3	50.811	0.603
90	69	9.2	2.8	14	0.1	125	0	75.360	0.580
91	88	9.1	3.3	19	0.2	74	0	52.027	0.554
92	76	9	3.1	13	0.1	65	6.2	66.308	0.587
93	77	8.5	3.1	10	0.1	31	3.2	31.290	0.609
94	80	9.2	2.7	15	0.1	67	0	72.836	0.568

Fig - 9

PATIENT	AGE	CAL	PHOS	ETWO	PROG	TALP	TINTES	PLIVER	SCORE
95	70	8.8	4	19	0.1	102	7.1	67.353	0.603
96	52	9.7	3.3	21	0.1	94	20.6	43.298	0.666
97	76	9.3	2.4	66	0.1	130	0	62.769	0.563
98	74	9.4	3.2	19	0.1	85	0	73.529	0.587
99	70	9.4	4	11	0.1	56	0	60.714	0.639
100	80	9.5	3.9	17	0.1	77	0	72.338	0.587
101	72	9.4	3.7	5	0.1	93	0	79.677	0.598
102	74	9.6	2	24	0.1	88	4.9	61.364	0.563
103	55	10.1	3.5	15	0.1	68	6	35.294	0.684
104	52	9.1	4.2	11	0.2	71	2.8	58.732	0.706
105	60	9.1	3.1	29	0.3	88	10.7	46.023	0.631
106	77	9.1	4	15	0.4	66	5.8	68.636	0.603
107	46	8.8	4	14	0.2	44	0	72.500	0.784
108	52	8.5	3.2	14	0.2	88	6.1	43.409	0.663
109	52	8.6	3.7	10	0.1	62	3.8	41.452	0.700
110	79	9.1	3.8	13	0.2	78	0	71.795	0.587
111	66	9.5	4.4	9	0.1	74	0	64.189	0.652
112	40	9.2	3.1	38	0.7	51	6.8	41.961	0.758
113	82	9.7	3.7	5	0.1	70	5.8	72.714	0.579
114	66	10	4.5	9	0.1	66	0	67.879	0.662
115	62	9.3	3	34	0.1	63	0	71.587	0.639
116	60	8.7	3.9	14	0.1	56	11.7	59.464	0.669
117	81	8.8	3.6	17	0.2	96	7.3	64.792	0.564
118	75	9.2	3.3	11	0.2	72	0	61.528	0.595
119	66	8.9	4.5	9	0.2	81	0	45.062	0.651
120	78	8.4	3.9	7	0.1	71	8.5	62.676	0.591
121	74	8.8	2.8	0	0.1	62	0	62.742	0.592
122	81	8.8	3.2	10	0.1	61	6.8	28.033	0.581
123	74	10.4	3.4	11	0.2	69	0	48.551	0.609
124	82	8.8	3.4	17	0.2	79	0	61.772	0.569
125	44	8.6	3.2	208	0.3	120	0	70.417	0.728
126	69	8.5	4.5	18	0.1	78	5.9	57.179	0.634
127	75	8.7	3.8	9	0.2	69	0	66.957	0.605
129	74	9	3.8	8	0.1	80	4	72.375	0.599
130	89	8.7	2.9	32	0.2	130	0	68.615	0.525
131	77	9.5	4.2	41	0.1	74	6.8	69.054	0.606
132	72	8.9	3.5	24	0.3	58	0	43.621	0.620
133	69	9.2	3.1	9	0.2	94	8.4	49.468	0.597
134	71	9	6.3	5	0.1	70	4.6	61.571	0.646
135	68	9.2	3.6	11	0.1	84	0	72.857	0.516
136	85	9	3.9	7	0.1	71	12.6	64.366	0.571
137	43	9.2	3.8	149	0.2	46	0	68.261	0.777
138	49	9.2	2.9	73	3.3	42	0	57.619	0.696
139	41	8.6	4.1	117	15.7	93	0	63.656	0.716
140	67	9	3.4	93	0.1	59	0	73.051	0.646
141	50	9.8	2.1	132	0.2	45	3.5	42.000	0.702
142	70	9.2	2.7	13	0.1	67	0	72.985	0.600

Fig_9

PATIENT	AGE	CAL	PHOS	ETWO	PROG	TALP	TINTES	PLIVER	SCORE
143	66	9.4	3.3	14	0.1	71	0	71.690	0.625
144	52	9	3.5	53	0.3	64	3.8	35.156	0.706
145	82	8.5	3.1	15	0.1	64	0	49.063	0.572
146	64	9.6	3.6	149	0.5	47	8.7	58.936	0.675
147	65	9.1	4.6	9	0.3	78	0	41.154	0.661
148	54	9.4	4.5	16	0.2	96	5.7	69.583	0.683
149	48	9.2	4.1	33	0.3	143	8.7	34.895	0.708
150	75	10	3.9	10	0.2	88	0	39.545	0.605
151	57	9.4	3.9	10	0.2	54	3.4	51.111	0.692
152	47	9.4	4	42	0.3	65	8.6	51.846	0.739
153	70	8.8	3.5	6	0.1	65	5	37.231	0.621
154	59	9.2	4	9	0.2	49	4.4	47.143	0.690
155	60	9.2	4.1	12	0.1	56	2.8	60.357	0.680
156	49	9.1	3.6	59	0.2	35	0	54.571	0.762
157	59	9.5	4.1	7	0.1	92	6.5	45.978	0.660
158	66	9.2	3.8	1	0.2	46	0	40.000	0.660
159	57	9.2	4.3	16	0.2	81	5.4	53.704	0.679
160	41	10	4.6	306	1.2	46	0	75.217	0.763
161	38	9.5	3.6	4	0.2	68	5.1	69.265	0.758
162	35	9.5	3.4	36	0.4	57	3.3	64.561	0.785

Fig_9

Testing Data for Osteoporosis Diagnostic System

PATIENT	AGE	CAL	PHOS	ETWO	PROG	TALP	TINTES	PLIVER	SCORE
123	52	9.4	4.2	-1	0.3	61	0	46	0.722
128	27	9.8	4.4	-1	0.4	58	0	43	0.862
131	39	9.1	3.9	156	11.5	44	0	59	0.820
173	26	9.5	3.8	41	0.2	98	0	60	0.790
201	30	9.1	3.5	123	3.3	90	0	53	0.814
206	39	9.2	2.6	50	1.5	74	89	42	0.729
213	46	9.7	3.4	37	0.2	90	0	36	0.708
220	46	8.8	3.4	118	4.3	90	9	63	0.996
223	49	9.7	3.3	72	0.4	78	8.6	38	0.717
234	38	9.5	3.2	186	12.6	50	0	46	0.803
243	33	9.3	4.4	65	0.3	58	0	40	0.857
244	53	9.6	3.7	96	0.3	58	0	49	0.725
245	30	9.5	3.2	-1	0.1	64	0	53	0.800
247	28	9.1	3.6	-1	0.3	58	0	50	0.821
249	22	9.3	3.5	42	0.2	98	11.1	38	0.782
253	24	9.2	3.4	-1	0.3	68	0	63	0.797
254	30	9.6	4	143	10.1	45	0	31	0.894
256	19	9.1	3.8	219	0.2	53	6.1	32	0.871
258	30	9.3	3.4	60	0.6	68	0	33	0.829
259	21	9.2	4.4	44	0.2	108	0	54	0.805
260	23	9.1	3.8	-1	0.3	62	0	61	0.818
261	23	9.4	3.4	-1	0.2	85	0	32	0.795
265	24	9.4	3.2	191	0.2	71	0	30	0.829
266	26	9	3.9	91	0.3	51	5.5	47	0.869
268	20	9.3	4.3	62	0.2	61	0	60	0.857
269	19	8.9	3.9	53	0.8	68	0	42	0.837
271	20	8.9	4.1	277	18.9	65	0	40	0.869
272	24	9	3.3	58	0.4	66	0	49	0.815
274	19	9.7	3.8	44	0.3	120	0	70	0.776
276	25	9.2	3.9	-1	0.9	65	5	27	0.837
279	21	10	4.6	115	0.5	106	0	70	0.834
280	21	9.5	4.1	202	0.9	68	0	57	0.856
281	20	9.1	3.6	-1	0.3	47	0	53	0.831
284	27	9	3.3	376	0.6	43	0	37	0.855
285	31	9.6	3.7	208	1.2	60	0	62	0.842
286	27	9	3.2	350	1.5	65	0	60	0.819
287	22	9	3.1	45	0.2	66	0	39	0.805
290	23	9.3	4.3	57	0.3	104	0	47	0.817
292	24	9.4	3.7	35	0.3	62	0	43	0.829
296	21	9.2	3.2	65	0.3	111	0	55	0.767
298	26	9.4	3.2	248	0.8	57	0	35	0.842
321	39	8.9	3.6	84	0.2	66	0	42	0.785
326	22	9.4	3.6	45	0.4	50	0	37	0.847
331	48	9.7	3.1	47	0.3	84	0	58	0.677
332	51	9.8	3.5	-1	0.3	64	0	40	0.707
333	53	9.2	3.3	166	1.2	63	0	56	0.705
340	42	9.1	2.8	52	0.2	67	0	64	0.728

Fig_10

PATIENT	AGE	CAL	PHOS	ETWO	PROG	TALP	TINTES	PLIVER	SCORE
342	50	9.6	3.8	76	0.3	62	0	71	0.730
346	44	9.2	3.4	111	4.9	72	12.4	50	0.977
347	41	9.5	3.7	-1	0.2	77	0	69	0.735
359	54	9.3	3.6	95	0.2	77	0	69	0.693
360	34	9.4	3.6	62	0.4	76	0	63	0.791
364	40	10	4.2	104	0.3	62	9	57	0.811
365	46	9	3.5	97	6.7	54	0	64	0.698
375	53	9.3	3.9	75	0.2	62	0	66	0.718
382	48	8.7	4.8	36	0.2	63	0	66	0.796
386	24	9.3	3.8	-1	0.3	68	0	65	0.812
389	22	9.1	4.3	-1	0.3	66	0	65	0.831
391	32	9.7	3.3	-1	0.4	57	0	64	0.797
393	29	9.6	3.5	30	0.5	47	0	61	0.828
394	27	8.9	4.1	-1	0.4	45	0	56	0.848
402	38	8.7	3.5	33	0.6	43	0	46	0.786
403	32	9.2	3.4	-1	0.3	21	0	24	0.842
404	49	9.2	4.5	-1	0.1	100	0	53	0.736
407	52	9.4	3.3	152	0.4	71	0	52	0.704
408	24	9	3.7	132	0.6	87	0	49	0.819
409	46	9.2	4.1	69	7.1	52	0	58	0.698
412	52	9.7	3.6	-1	0.2	68	0	29	0.704
416	44	9.7	3.4	142	0.7	80	0	79	0.692
417	49	9.5	3.4	-1	0.2	75	0	47	0.708
418	49	10	4.2	403	1.3	88	0	75	0.678
419	26	9.3	3	-1	0.4	82	0	65	0.767
422	38	9.2	3.3	198	0.2	76	0	24	0.780
539	46	9.3	4.5	793	0.7	46	0	33	0.813
545	26	9.7	3	96	0.3	65	0	42	0.825
549	26	9.5	3.9	50	0.4	46	7.2	49	0.862
560	33	8.9	3.4	118	0.3	79	7.4	61	0.791
562	23	9.4	3.2	-1	0.4	58	0	36	0.815
579	20	9	3.4	48	0.5	80	0	53	0.797
588	36	9.3	3.5	-1	0.1	48	0	51	0.793
591	28	9.7	3.7	36	0.5	52	0	54	0.838
593	35	9	3.7	108	0.3	69	9.6	41	0.811
597	32	9.3	3.5	-1	0.3	53	0	71	0.801
602	49	9.3	3.6	180	4.3	69	0	46	0.693
2520	29	9.2	4.4	52	0.2	49	0	41	0.877
3000	28	9.4	3.7	49	0.4	66	0	53	0.828
3020	25	9.6	4	164	16.8	56	0	41	0.880
100	52	8.7	3.1	371	0.3	59	0	54	0.703
113	53	9.8	3.5	3	0.2	63	0	67	0.690
126	42	9	4	99	16.4	83	14.4	36	0.984
130	50	9.3	3.5	336	0.6	40	0	25	0.754
139	74	8.9	3.7	209	0.4	35	5.9	36	0.648
160	60	9.5	3.9	81	0.3	47	5.2	53	0.704
176	56	9	2.9	115	0.2	64	0	58	0.677

Fig. 10

PATIENT	AGE	CAL	PHOS	ETWO	PROG	TALP	TINTES	PLIVER	SCORE
177	39	9.4	3.1	71	0.2	82	0	45	0.751
208	42	9.1	2.4	177	15.6	54	0	70	0.757
211	50	9.5	3.1	69	0.2	77	0	60	0.696
214	25	9.2	3.7	-1	0.9	43	0	29	0.852
251	24	9.5	3.9	-1	0.1	61	0	25	0.844
273	30	9.4	3	99	0.2	156	8.3	26	0.776
299	27	9.2	3.2	41	0.2	77	0	58	0.788
309	35	9	3.9	55	0.3	90	8.7	62	0.773
316	41	9.4	3.5	75	7.3	44	0	27	0.733
320	52	9.3	3.4	57	0.2	58	0	55	0.707
355	54	9.1	3.6	93	0.4	55	0	40	0.718
358	54	9.5	3.6	38	0.3	71	0	54	0.688
361	49	9	3.8	31	0.3	74	9.5	50	0.744
371	49	9.5	3.8	-1	0.2	72	10.3	42	0.698
377	52	9.6	3.6	120	0.2	68	0	53	0.717
385	55	9	3.3	-1	0.2	97	0	73	0.646
398	53	9.3	4.6	30	0.2	97	10.3	28	0.697
406	26	9.6	3.4	35	0.3	62	0	70	0.806
414	52	9.3	3.9	56	0.4	66	0	72	0.710
421	31	9	3.5	72	0.3	69	0	52	0.818
536	48	9.4	3.4	54	3.2	56	0	63	0.688
544	38	9.3	2.4	126	0.2	64	0	59	0.747
566	36	9.3	4	-1	0.5	41	0	44	0.822
603	27	8.9	4.4	42	0.3	48	0	38	0.872
109	54	9.4	3.3	64	0.3	121	9.6	65	0.652
110	58	9.4	3.9	20	0.1	85	0	58	0.663
129	45	8.8	3.7	197	10.8	56	0	53	0.733
141	46	9.7	3.6	141	2.1	68	0	44	0.709
158	45	8.9	3.3	243	0.8	63	0	43	0.763
164	51	9.3	3.5	139	0.5	59	0	43	0.727
170	51	9.4	3.3	120	4.2	50	0	30	0.737
178	36	9.7	3.3	139	0.4	40	0	46	0.824
180	38	8.7	3.4	48	0.4	52	0	39	0.783
181	34	8.7	3.4	78	11.1	67	6.8	33	0.817
183	44	9	3.6	131	0.2	35	0	54	0.807
191	45	9.3	3.5	-1	0.4	45	0	58	0.760
194	29	9.2	3.7	89	0.1	82	0	52	0.824
200	45	9.2	3.7	226	1.6	46	0	27	0.786
204	25	9.5	3.5	170	0.3	43	0	46	0.863
209	42	9.6	2.9	539	0.4	50	0	64	0.738
216	46	9.2	3.6	156	0.5	43	0	39	0.780
218	43	9.5	4.4	133	0.7	59	0	41	0.791
225	37	9	2.7	159	1.4	64	6.7	48	0.766
226	72	9.9	4.9	46	0.3	77	8.9	61	0.643
227	46	9.6	4.4	-1	0.2	94	0	38	0.757
230	43	9.3	3.4	94	0	71	0	77	0.721
231	35	8.9	3.2	-1	0.3	51	0	61	0.777

Fig_10

PATIENT	AGE	CAL	PHOS	ETWO	PROG	TALP	TINTES	PLIVER	SCORE
233	50	9.1	3.5	43	0.3	73	0	23	0.709
238	46	8.3	3.4	234	0.2	55	5.9	46	1.094
239	40	9.5	3.6	43	1.6	71	0	63	0.758
246	34	9.8	3.3	-1	0.3	42	0	58	0.804
248	51	8.9	3.8	76	0.2	61	0	62	0.724
255	19	9.6	4.3	49	0.3	86	0	34	0.841
257	23	8.9	2.9	118	2.7	75	0	21	0.816
262	26	9.1	3.6	-1	0.2	47	0	24	0.844
267	23	9	3.8	170	0.4	84	0	50	0.825
270	21	9.1	3.9	47	0.9	40	0	70	0.850
277	27	9.4	4.6	58	0.2	138	0	69	0.811
278	29	9.4	3.9	404	1.2	62	7.9	46	0.864
282	28	9.4	3.9	60	0.2	57	0	36	0.859
283	25	10	3.6	-1	0.3	48	0	32	0.847
288	24	9.7	4.1	65	0.3	107	7.2	35	0.816
289	27	9.4	3.4	68	0.6	87	0	37	0.812
291	26	9.6	3.3	-1	0.3	58	0	28	0.823
293	26	9.6	4	32	0.2	68	0	35	0.838
295	26	9.1	3.3	57	0.5	51	0	63	0.324
310	47	9.5	3.6	306	1.5	53	0	46	0.730
314	48	9.1	2.7	-1	0.2	64	0	53	0.704
328	37	9.4	3.2	54	0.4	74	0	55	0.762
335	47	9.2	4.2	49	0.4	74	0	56	0.745
341	37	9.3	3.3	-1	0.1	68	0	56	0.758
349	50	8.8	3.1	151	0.1	67	8	30	0.710
353	50	9	4.1	374	0.2	53	0	30	0.759
354	58	9.7	4.3	74	0.3	48	6.8	45	0.726
363	50	9.1	3.3	76	0.6	93	13.8	31	0.694
366	24	9.4	2.7	-1	0.5	50	0	31	0.806
372	21	10	2.9	56	0.7	79	0	61	0.789
378	42	9.2	3.5	169	0.1	72	0	56	0.754
381	62	9.5	3.6	56	0.1	63	0	30	0.672
384	62	9.4	4	34	0.2	98	25.3	33	0.644
387	52	9.6	3.7	38	0.2	133	0	55	0.669
388	64	9.1	3.1	87	0.3	80	0	75	0.636
396	57	8.8	3.1	76	0.2	83	7.7	51	0.659
397	58	9.6	3.3	73	0.3	85	0	52	0.665
400	50	9.1	3.5	-1	0.2	99	9.7	50	0.671
410	37	9.5	2.8	-1	0.3	44	0	48	0.769
411	49	9.4	3.1	138	7.5	69	0	27	0.674
415	31	9.6	4.6	-1	0.4	42	0	72	0.861
424	54	9.4	3.1	59	0.4	101	0	35	0.662
507	45	9	3.4	46	0.3	55	0	65	0.745
513	25	9.4	3.5	52	0.2	72	0	52	0.815
533	61	9.6	3.9	-1	0.1	80	0	61	0.655
535	24	9	3.3	-1	0.3	68	0	55	0.795
537	48	9.9	3.2	32	0.3	81	0	58	0.679

Fig_10

PATIENT	AGE	CAL	PHOS	ETWO	PROG	TALP	TINTES	PLIVER	SCORE
540	45	9.5	2.9	391	0.7	49	7	35	0.955
541	54	9.3	4.4	-1	0.2	68	0	37	0.714
542	32	9.2	3.4	80	0.6	51	0	60	0.829
546	62	9.3	4.5	100	0.1	45	0	59	0.711
548	52	9.2	4.1	-1	0.4	55	0	59	0.719
550	48	9.9	3.7	92	0.3	127	0	78	0.668
554	36	9.1	3.5	42	0.4	44	0	37	0.808
561	30	8.9	3	54	0.2	55	0	54	0.809
568	53	9.6	3.2	-1	0.3	49	0	40	0.699
569	26	9.2	4.5	274	0.5	80	9	30	0.870
571	45	9.1	3.3	402	1	72	0	54	0.731
572	58	9.5	3.9	52	0.2	57	7.3	23	0.700
576	65	9.5	3.3	43	0.2	78	0	56	0.632
580	50	9.3	3.2	60	0.2	76	8	43	0.696
582	44	8.9	3	119	0.2	80	7.1	24	0.967
583	45	8.9	3.2	353	0.2	68	0	64	0.744
586	41	8.8	3.7	223	0.7	73	12.4	22	1.206
589	52	9.3	3.5	-1	0.3	88	0	45	0.678
592	55	9.2	3.7	95	0.3	50	0	57	0.717
595	46	9.1	3	128	4.5	90	8.2	35	1.000
596	46	9.5	3.6	-1	0.2	53	0	47	0.757
604	56	9.3	4.3	-1	0.2	84	0	53	0.684
605	47	8.9	3.4	-1	1.4	64	0	40	0.740
608	46	9.7	3.8	-1	0.1	69	0	48	0.742
2500	30	9.2	3.6	70	0.2	62	10.1	42	0.842
3010	24	9.4	3.8	178	15.9	33	0	34	0.894
106	52	9.1	3.4	44	0.2	86	9	55	0.674
117	61	9.5	3.2	-1	0.1	85	0	60	0.634
121	46	9.5	2.9	48	0.3	80	0	54	0.690
133	47	9	4.2	203	15	57	7	52	0.939
151	36	9.1	3.8	-1	0.3	80	0	57	0.768
156	34	9.6	3.9	114	17.9	41	0	37	0.866
166	51	9.2	3.8	60	0.1	60	0	42	0.727
167	49	8.5	3.8	76	0.5	97	10	48	0.810
179	32	9.5	3.6	59	0.2	61	0	47	0.826
182	51	8.4	3.7	99	0.2	84	0	31	0.709
189	53	9.2	4.2	-1	0.1	68	8	47	0.705
212	37	9.4	3.7	162	18.6	55	0	57	0.818
217	67	9.4	3.2	-1	0.2	64	6.3	33	0.628
219	50	9.6	3.7	-1	0.3	81	0	18	0.707
222	56	9.1	3.9	30	0.2	102	0	61	0.657
228	51	9.4	3.5	141	0.4	61	0	28	0.730
235	41	9	4.3	96	0.3	45	0	39	0.868
252	52	9.5	3.3	34	0.2	79	0	48	0.681
264	28	9.1	3.4	-1	0.2	50	0	60	0.816
275	25	9.1	2.5	-1	0.4	38	0	34	0.806
297	23	9.4	4.4	177	12.2	72	0	55	0.873

Fig_10

PATIENT	AGE	CAL	PHOS	ETWO	PROG	TALP	TINTES	PLIVER	SCORE
317	47	9.6	2.4	-1	0.2	60	0	27	0.709
324	46	9.4	4	117	0.4	75	0	54	0.732
327	46	9	3.4	322	1	46	0	52	0.765
329	49	9.5	3.7	72	2.4	78	0	45	0.692
337	46	9.3	3.5	122	10.3	48	0	42	0.700
352	56	8.8	3.6	73	0.2	53	0	35	0.707
357	35	9.4	2.9	89	2.3	71	7.6	23	0.791
367	42	9.1	3.2	58	0.5	90	9.8	64	0.846
369	62	9.6	3.6	31	0.2	86	0	39	0.645
379	42	8.6	3.4	156	6.3	84	0	61	0.742
380	49	9.6	4.2	-1	0.2	71	11	52	0.690
383	62	9	3.5	81	0.1	66	7	52	0.665
392	63	9.5	3.4	-1	0.3	98	0	76	0.620
517	25	10	4	31	0.1	50	0	50	0.853
526	47	9.1	3.8	94	0.2	62	0	51	0.750
543	59	9.4	4.2	-1	0.2	104	0	20	0.662
553	48	8.8	4.1	35	0.5	57	0	66	0.759
556	69	9.6	4	77	0.1	44	6.8	53	0.668
567	29	9.4	4.2	-1	0.3	67	9.7	33	0.841
575	45	9.1	3.1	406	0.6	104	0	54	0.704
584	57	9.2	3.2	141	0.2	79	0	40	0.674
599	49	8.9	4	42	0.2	79	9	51	0.748
2510	26	9.5	3.9	157	16.2	56	0	25	0.881
105	75	9.4	4.1	42	0.4	88	8	52	0.605
116	67	8.9	4	-1	0.3	56	0	70	0.647
118	55	8.9	4.3	38	0.2	78	0	37	0.699
119	56	9.3	4.3	75	0.3	41	0	43	0.740
127	73	9	4.1	30	0.4	54	6.5	35	0.632
134	50	9.4	3.7	486	0.4	54	0	63	0.737
136	36	9.1	3.6	167	5.7	154	0	74	0.758
140	56	9	3.9	-1	0.2	94	0	58	0.663
143	55	9.1	4	-1	0.2	65	0	38	0.698
145	49	9.3	3.3	67	8.9	63	0	54	0.656
147	53	9.1	3.6	75	0.2	103	4.9	78	0.670
157	52	9.2	3.5	65	0.2	70	0	62	0.701
159	57	9.4	3	-1	0.2	129	30.2	27	0.631
161	74	9.3	3.9	-1	0.1	62	0	34	0.623
162	62	9	4.1	81	0.1	54	7.2	21	0.698
163	46	9.2	2.9	128	5.7	62	0	35	0.704
165	57	9.2	2.9	-1	0.2	108	0	39	0.629
169	59	9.5	3.9	61	0.1	64	0	34	0.693
174	40	9.5	3.2	261	4.6	85	0	22	0.764
175	39	9.1	2.3	126	3.5	90	0	36	0.726
185	78	8.7	2.9	-1	0.1	99	8.5	35	0.563
186	73	9.2	2.9	62	0.2	55	0	42	0.616
187	59	9	3.8	55	1.8	70	0	37	0.682
190	41	8.7	3	110	11.6	60	0	26	0.772

Fig_ 10

PATIENT	AGE	CAL	PHOS	ETWO	PROG	TALP	TINTES	PLIVER	SCORE
192	42	9.5	4.1	46	0.4	58	0	66	0.767
195	58	9	3.4	236	0.1	61	0	40	0.688
202	67	9.4	3.2	66	0.3	59	0	61	0.641
210	60	9.6	3.2	82	0.1	49	4.7	56	0.681
215	45	9.2	3.8	135	1.8	70	0	56	0.729
221	52	9.5	4	83	0.8	60	0	57	0.735
224	53	8.7	3.6	110	0.2	52	0	27	0.726
229	51	9.5	4.1	33	0.3	97	0	51	0.693
237	59	9.4	4	-1	0.2	90	9.9	34	0.660
241	48	9.6	3.9	44	0.5	68	0	31	0.747
263	23	9.6	4.2	39	0.5	69	0	28	0.853
294	25	9.3	3.8	91	2.9	64	0	54	0.853
308	54	8.8	3.1	-1	0.2	40	0	48	0.692
313	53	9.8	4.3	-1	0.4	90	0	40	0.700
319	31	9	3.5	296	2.8	49	0	27	0.859
322	42	9.4	3.3	-1	0.3	63	6.6	41	0.757
323	56	8.8	3.6	38	0.1	54	6.5	54	0.685
334	68	8.8	4.1	83	0.4	44	0	39	0.676
338	42	9.9	3.2	35	1	92	9.4	41	0.719
339	52	9.9	3.7	84	0.2	78	0	62	0.711
343	44	9.1	3.4	55	1.3	75	8	55	0.834
344	61	9.2	4.3	82	0.3	116	8.3	65	0.655
351	46	9.2	3.9	140	0.3	58	8	51	0.905
362	53	9.6	3.9	94	0.1	83	0	72	0.703
373	62	9.9	3.4	172	0.2	83	0	66	0.654
376	70	9.9	4.7	-1	0.1	109	0	38	0.627
390	72	9	3.8	-1	0.2	108	14.5	28	0.597
395	54	9.2	4.1	-1	0.3	96	0	67	0.674
399	49	9.4	3	-1	0.4	64	0	50	0.701
401	83	8.7	3.8	31	0.2	111	0	55	0.558
413	46	9.4	3.6	90	0.1	98	25.1	54	0.746
423	44	9.2	3.6	62	0.2	47	0	45	0.785
527	38	9.3	3.3	40	0.4	62	4.1	56	0.763
552	54	9.4	3.7	-1	0.2	80	0	57	0.679
555	50	9.5	3	108	0.2	126	7.4	50	0.670
557	59	9.4	3.5	192	0.2	42	0	66	0.697
563	44	9.5	3.8	37	0.5	46	0	39	0.790
565	70	9	3.2	-1	0.2	54	0	45	0.623
570	24	9.2	3.5	50	0.5	53	7	32	0.842
573	65	9.9	4.2	-1	0.3	59	0	56	0.665
574	65	9.2	3.2	122	0.3	64	0	58	0.649
577	46	9.5	4.1	397	.04	56	0	70	0.738
585	62	9	3.5	56	0.2	77	0	21	0.658
590	65	9.2	3.7	-1	0.2	72	0	57	0.640
594	55	9.9	3.6	-1	0.2	43	0	52	0.705
601	45	8.9	3.1	81	3.4	75	6.6	54	0.858
607	81	9.7	3.5	-1	0.1	66	0	31	0.590

Fig_10

PATIENT	AGE	CAL	PHOS	ETWO	PROG	TALP	TINTES	PLIVER	SCORE
111	69	9	3.3	34	0.4	68	9.3	55	0.615
124	51	9.2	3.7	35	0.2	69	0	48	0.704
149	52	9.2	3.9	-1	0.3	82	0	72	0.686
152	56	9	2.5	79	0.2	77	0	43	0.659
168	56	9.1	4.1	-1	0.1	76	0	45	0.686
196	61	9.3	3.3	69	0.1	97	8.8	36	0.642
330	63	8.9	3.8	-1	0.1	68	9.6	54	0.648
345	65	8.9	4.2	40	0.1	50	5.3	19	0.676
356	53	9.7	3.5	54	0.2	99	0	60	0.673
534	66	8.9	3.5	78	0.1	61	0	53	0.654
551	67	9.3	3.5	47	0.2	66	10	47	0.635
558	38	9.3	3.3	137	7.9	85	0	66	0.765
578	51	8.8	3.9	34	0.3	50	0	54	0.722
581	53	9.3	3.4	62	0.2	56	0	54	0.707
104	63	9.3	4.3	77	0.3	88	7.6	41	0.669
120	66	9.3	3.8	35	0.2	81	0	61	0.633
125	63	9.3	4	-1	0.4	92	0	55	0.642
138	60	9.3	2.8	58	0.3	84	0	77	0.634
144	68	9.5	4.6	-1	0.4	118	0	30	0.632
146	67	9.8	3.3	-1	0.2	74	0	59	0.623
150	44	9.6	3.8	65	0.2	122	0	44	0.716
193	50	10	3.2	-1	0.3	67	0	24	0.705
198	36	9.4	3.2	262	0.6	101	14.1	18.3	0.761
207	57	9.7	3.5	33	0.3	74	0	57	0.668
232	65	9.4	4	80	0.2	69	0	53	0.668
236	48	9	3.6	73	8.9	43	0	33	0.704
240	53	9.1	4.1	41	0.2	56	5	39	0.721
242	57	9.4	3.8	-1	0.1	71	9.8	34	0.677
315	64	9.7	3.5	46	0.2	83	11.4	37	0.639
318	49	9.8	3.2	168	3	46	0	34	0.701
325	57	9.2	4.3	-1	0.1	124	0	43	0.662
336	55	9.2	3.6	-1	0.3	76	10	28	0.676
348	58	8.7	3.5	-1	0.2	55	0	55	0.672
368	62	9.4	3.9	-1	0.2	68	0	45	0.663
370	77	9.5	4.1	-1	0.4	98	10.2	21	0.596
374	76	9.9	3.7	-1	0.3	78	0	54	0.600
528	80	9	3.9	6	0.2	103	0	71	0.571
529	66	9.1	3.5	-1	0.1	67	0	54	0.634
538	79	9.4	3.9	-1	0.3	96	9.4	39	0.582
547	73	9.3	3.6	126	0.1	64	0	22	0.636
559	68	9.7	3.6	91	0.1	37	0	57	0.669
564	80	9.5	3.6	33	0.2	113	10.7	52	0.565
598	69	9.6	4.4	128	0.7	72	2.5	57	0.660
600	64	9.2	4.5	-1	0.4	66	0	47	0.671
606	78	9.5	3.9	-1	0.1	77	0	58	0.596

Fig_10

```
**********************************
** Program  : Calc. prg
** Author   : Terminal solutions (JJK)
** Date     : 14 August 1992
** Purpose  : Quios calculating program
**********************************
set decimal to 16
set fixed on
****** Set Coefficient information to valiables ******
store    0.000000000  to outnumb
store  - 1009.33 to constant
store  - 0.014786248        to c _age
store    424.462000000      to c_ cal
store  - 66.3468            to c_cal2
store  - 0.025761569        to c_csage
store  - 0.019715699        to c_csphos2
store    0.027232306        to c_cstintes
store  - 0.000000263        to c_etwoimx2
store  - 12.0319            to c_ phos
store    7.499334732        to c_ phos2
store  - 2.220366330        to c_ phos3
store    0.313008013        to c_ phos4
store  - 0.016909117        to c_ phos5
store    0.001369629        to c_ prog2
store  - 0.000428831        to c_ progpliv
store    0.001798351        to c_ progtint
store    0.001841052        to c_ tnetwoimx
store    0.007649946        to c_ tntintes
store    0.002153604        to c_ agephos
store    4.606320653        to c_ cal3
store  - 0.119876531        to c_ cal4
store  - 0.011786744        to c_ talp
store  - 0.000170224        to c_talp2
store    0.000000706        to c_talp3
outnumb= (constant)
outnumb=outnumb   + (c_age  * age)
outnumb=outnumb   + (c_ cal  * cal)
outnumb=outnumb   + (c_ cal2  * (cal  * cal))
outnumb=outnumb   + (c_ csage * (cos(age)))
outnumb=outnumb   + (c_ csphos2 * (cos(phos * phos)))
outnumb=outnumb   + (c_ cstintes *  (cos(tintes)))
outnumb=outnumb   + (c_ etwoimx2 * (etwoimx   * etwoimx))
outnumb=outnumb   + (c_ phos  *  phos)
outnumb=outnumb   + (c_ phos2  *  (phos  *  phos))
outnumb=outnumb   + (c_ phos3  *  (phos  *  phos. *  phos))
outnumb=outnumb   + (c_ phos4  *  (phos  *  phos  *  phos  *  phos))
outnumb=outnumb   + (c_ phos5  *  (phos  *  phos  *  phos  *  phos  *  phos))
outnumb=outnumb   + (c_ prog2  *  (prog  *  prog))
outnumb=outnumb   + (c_ progpliv  *  (prog  *  pliv))
outnumb=outnumb   + (c_ progtint  *  (prog  *  tintes))
outnumb=outnumb   + (c_ tnetwoimx  *  (tan (etwoimx)))
outnumb=outnumb   + (c_ tntintes  *  (tan(tintes)))
outnumb=outnumb   + (c_ agephos  *  (age  *  phos))
outnumb=outnumb   + (c_ cal3  *  (cal  *  cal  *  cal ))
outnumb=outnumb   + (c_ cal4  *  (cal  *  cal  *  cal  *  cal))
outnumb=outnumb   + (c_ talp  *  talp)
outnumb=outnumb   + (c_ talp2  *  (talp  *  talp))
outnumb=outnumb   + (c_ talp3  *  (talp  *  talp  *  talp))
```

Fig_11

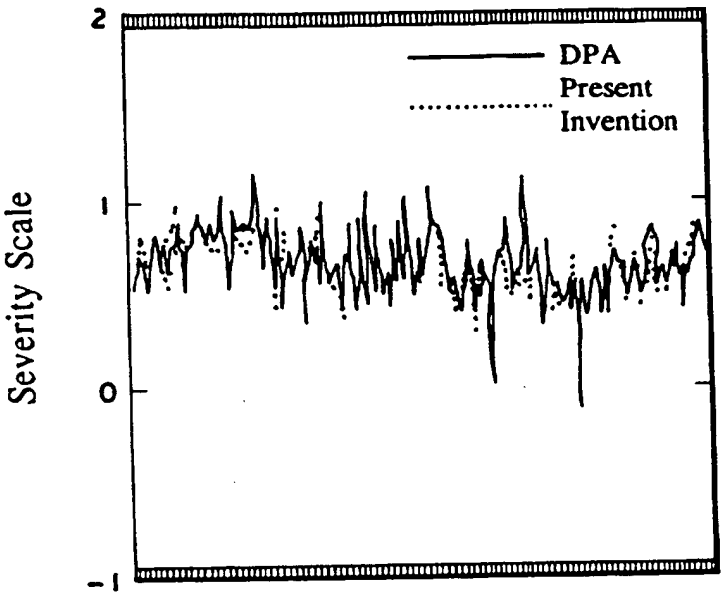


Fig_12

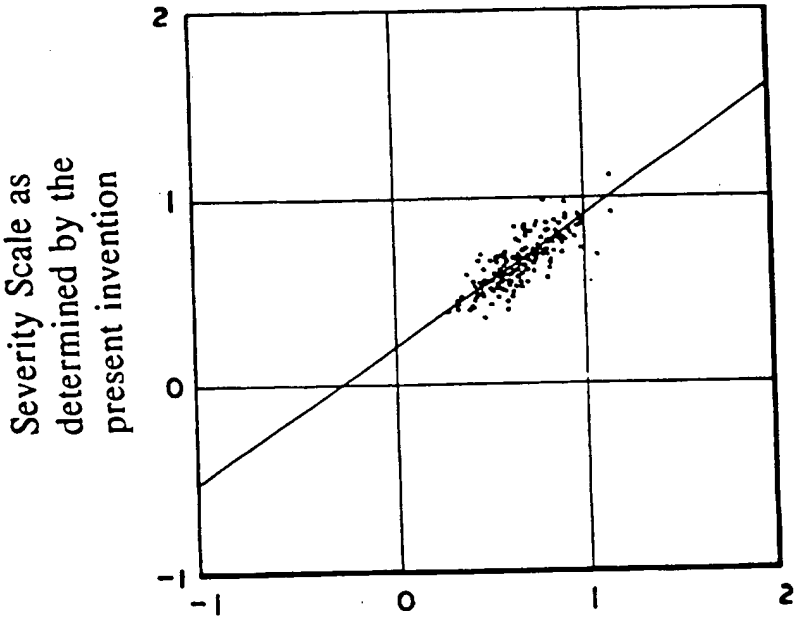


Fig_13

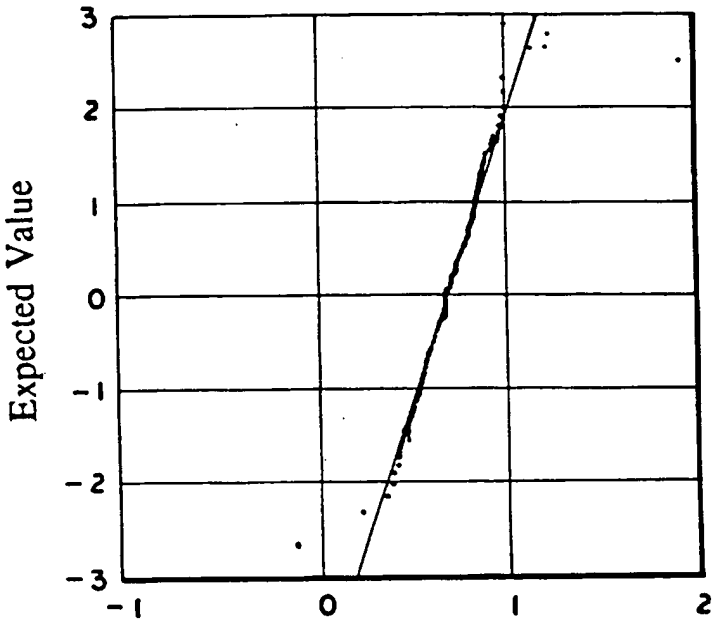


Fig_14

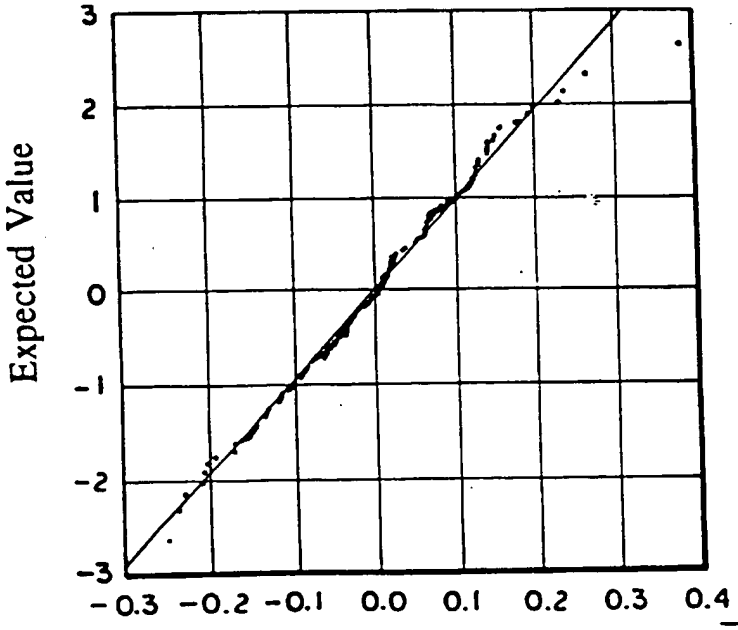


Fig_15

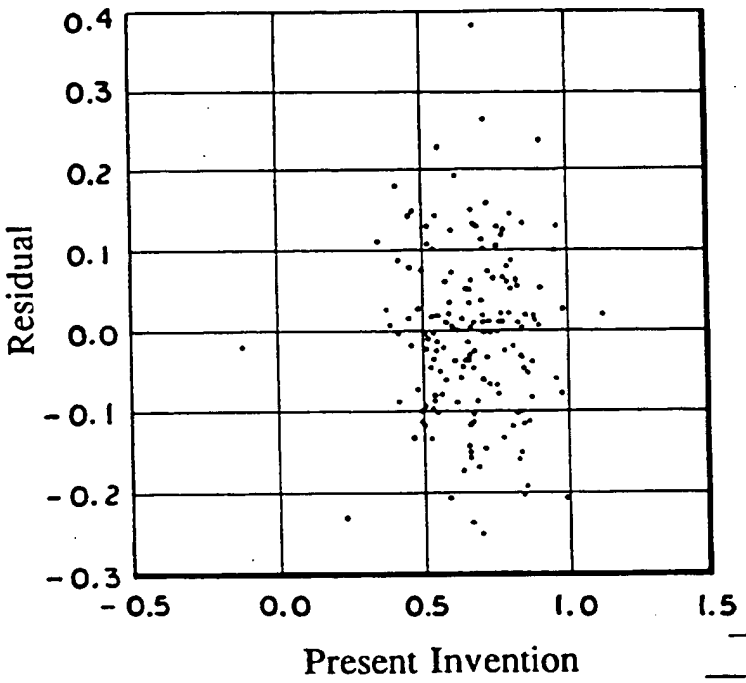


Fig_16

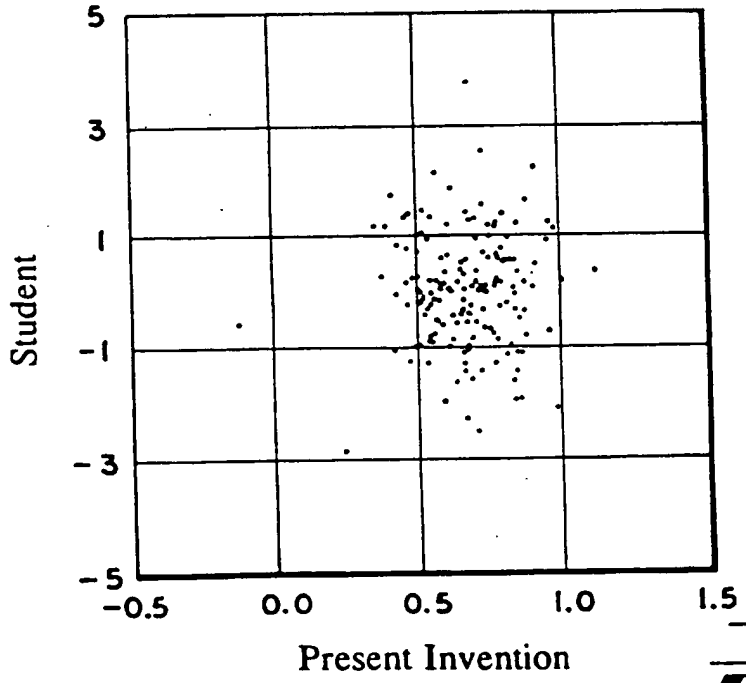


Fig_17

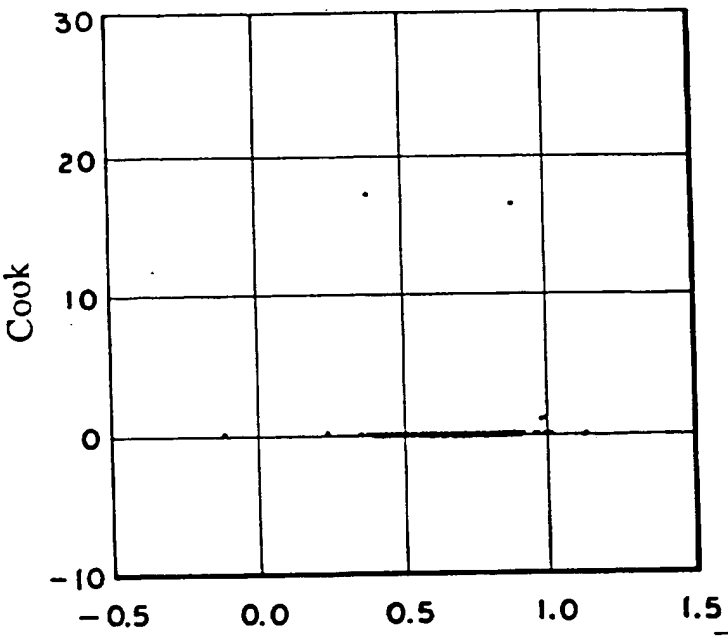


Fig_18

Prostate Cancer Prognosis Training Data

nTPS	nPSA	PAP	CKA	Testosterone	Stable	Progress	ANNoutput1	ANNoutput2	Actual	Index
0.53	0.69	7.8	1.3	5.6	0.1	0.9	0.10	0.90	0.9	0.90
0.55	0.59	0.7	0.4	1.9	0.9	0.1	0.90	0.10	0.1	0.10
0.8	0.44	0.6	1	0.3	0.9	0.1	0.90	0.10	0.1	0.10
0.6	0.02	1.3	1.2	1.6	0.9	0.1	0.89	0.11	0.1	0.11
0.92	0	1.2	1.7	1.2	0.9	0.1	0.90	0.10	0.1	0.10
0.3	0.02	1.5	0.8	0.5	0.9	0.1	0.90	0.10	0.1	0.10
0.62	0.04	0.7	0.6	0.8	0.9	0.1	0.90	0.10	0.1	0.10
1.13	0	0.4	0.9	1.1	0.9	0.1	0.90	0.10	0.1	0.10
1.66	0.01	0.5	0.9	1	0.9	0.1	0.90	0.10	0.1	0.10
0.8	0.26	1.6	0.6	0.3	0.9	0.1	0.89	0.11	0.1	0.11
0.82	0.42	1.1	1.2	0.9	0.9	0.1	0.90	0.10	0.1	0.10
0.81	0.44	2.9	0.4	0.1	0.1	0.9	0.10	0.90	0.9	0.90
0.68	0.66	5.5	0.2	0.3	0.1	0.9	0.10	0.90	0.9	0.90
0.68	0.22	3	0.9	2.9	0.1	0.9	0.10	0.90	0.9	0.90
0.55	0.02	1.4	1	0.7	0.9	0.1	0.90	0.10	0.1	0.10
0.5	0.01	0.5	0.7	2	0.9	0.1	0.90	0.10	0.1	0.10
0.31	0.02	0.5	10	4.3	0.9	0.1	0.90	0.10	0.1	0.10
0.66	0.44	2.6	1.2	4.1	0.1	0.9	0.17	0.83	0.9	0.83
1.6	0.02	2.6	4.8	0.4	0.1	0.9	0.11	0.89	0.9	0.89
1.99	0.01	1.9	5.4	0.5	0.1	0.9	0.11	0.89	0.9	0.89
0.66	0.44	2.6	1.2	4.1	0.9	0.1	0.88	0.12	0.1	0.12
0.75	0.93	29.2	0.2	5.1	0.1	0.9	0.10	0.90	0.9	0.90
1.04	1.76	77	1.2	7	0.1	0.9	0.10	0.90	0.9	0.90
0.56	1	8.1	0.4	0.9	0.9	0.1	0.90	0.10	0.1	0.10
0.8	0.86	7.3	2.3	0.4	0.9	0.1	0.90	0.10	0.1	0.10
0.47	0.37	3.3	1	5.1	0.1	0.9	0.10	0.90	0.9	0.90
0.49	0.02	1.7	0.6	0.2	0.9	0.1	0.90	0.10	0.1	0.10
0.59	0.02	0.9	0.4	0.2	0.9	0.1	0.90	0.10	0.1	0.10

Fig_19

Prostate Cancer Prognosis Training Data

0.44	0.02	1	0.3	0.3	0.9	0.1	0.90	0.10	0.1	0.10
0.45	0.01	1	0.1	0.3	0.9	0.1	0.89	0.11	0.1	0.11
0.89	0.01	0.6	1.1	0.6	0.9	0.1	0.89	0.11	0.1	0.11
1.73	1.28	12.1	5	3.8	0.1	0.9	0.10	0.90	0.9	0.90
1.83	0.15	5.1	1.8	0.4	0.9	0.1	0.90	0.10	0.1	0.10
0.82	0.01	0.4	0.5	0.1	0.9	0.1	0.90	0.10	0.1	0.10
0.62	0.04	0.7	0.6	0.8	0.9	0.1	0.90	0.10	0.1	0.10
0.35	0.01	1	0.6	1	0.9	0.1	0.90	0.10	0.1	0.10
1.06	0.43	3.8	1	4.6	0.1	0.9	0.10	0.90	0.9	0.90
0.25	0.04	0.3	0.7	0.4	0.9	0.1	0.90	0.10	0.1	0.10
0.11	0.03	1.8	1.5	0.5	0.9	0.1	0.90	0.10	0.1	0.10
0.74	0.01	0.2	0.5	0.1	0.9	0.1	0.89	0.11	0.1	0.11

Fig_19

Prostate Cancer Prognosis Testing Data

nTPS	nPSA	PAP	CKA	Testosterone	Stable	Progress	NNoutput1	NNoutput2	Actual	Index
0.39	0.15	2.3	1	1.4	0.9	0.1	0.89	0.11	0.1	0.11
0.9	0.05	2	1.3	0.6	0.9	0.1	0.90	0.10	0.1	0.10
0.43	0.29	1.9	2.2	0.8	0.9	0.1	0.90	0.10	0.1	0.10
0.63	0.37	3.4	1	5	0.1	0.9	0.10	0.90	0.9	0.90
0.52	0.01	0.3	1.8	0.1	0.9	0.1	0.88	0.12	0.1	0.12
1.33	0.42	0.1	1.1	0.6	0.1	0.9	0.77	0.23	0.9	0.23
0.76	0.6	51.7	0.5	3.9	0.1	0.9	0.10	0.90	0.9	0.90
0.64	0	1.1	2.3	0.7	0.9	0.1	0.88	0.12	0.1	0.12
0.3	0.05	1.1	2.2	0.7	0.9	0.1	0.87	0.13	0.1	0.13
1.14	1.01	1.3	1	0.5	0.9	0.1	0.89	0.11	0.1	0.11
0.7	0.03	0.6	0.1	1.5	0.9	0.1	0.90	0.10	0.1	0.10
0.42	0.25	2.2	0.9	6.6	0.9	0.1	0.81	0.19	0.1	0.19

Fig_19

N 4 i = 0_N-1 4 input nodes

N1 8 j = 0_N1 1 8 neurons in first hidden layer

N2 1 1 output neurons

f(x) $\frac{1}{1+e}$ Logistic neuron output function

$$\text{Imax.} = \begin{vmatrix} 100 \\ 25 \\ 40 \\ 40 \end{vmatrix} \quad \text{Imin.} = \begin{vmatrix} 0 \\ 10 \\ 0 \\ 0 \end{vmatrix} \quad \text{Irange.} = \text{Imax.} - \text{Imin.}$$

W1 = READPRN (link 1_wgt)

W2 = READPRN (link 2_wgt)

W1 =

-0.26	-0.33	-0.15	-0.44	0.02
0.03	0.47	0.09	0.05	-0.17
0	-1.16	-0.07	-0.12	-0.05
0.06	1.25	-0.26	0.22	-0.09
0.01	0.73	0.21	0.15	0.02
0.02	0.74	-0.1	0.21	0.07
0.23	1.18	0.05	-0.63	0.18
0.31	1.25	0.27	0.01	0.07

9x(5+1) weitht matrix between input layer and the first hidden layer

W2 = (0.43 1.46 1.39 3.91 3.96 2.16 2.25 -6.36 -4.1) 2x9 weight matrix between hidden layer and output layer

Fig_20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01379

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/53, 33/573, 33/574; G06F 159:00
US CL :364/413.01; 435/7.1, 7.23; 436/518, 536

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 364/413.01; 435/7.1, 7.23, 7.4, 7.92; 436/506, 518, 528, 536, 64, 74, 105

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US, A, 5,130,936 (SHEPPARD ET AL) 14 July 1992, see entire document, especially column 6.	2 ----- 1,3-5
Y	DIANON SYSTEMS, issued June 1992, "Physician Participation Program ONCOSITE™ Biomarker Tests", see entire document.	1, 3-5
Y	WO, A, 93/12255 (BARNHILL) 24 June 1993, see entire document.	1, 3-5
X	ANNUAL INTERNATIONAL CONFERENCE OF THE IEEE ENGINEERING IN MEDICINE AND BIOLOGY SOCIETY, Volume 12, Number 3, issued 1990, J.H. Frenster, "Neural Networks for Pattern Recognition in Medical Diagnosis", pages 1423-1424, see entire document.	1-5

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
20 APRIL 1995	22 MAY 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JAMES L. GRUN, PH.D. Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01379

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AMERICAN JOURNAL OF CLINICAL PATHOLOGY, Volume 96, Number 1, issued July 1991, J.W. Furlong et al, "Neural Network Analysis of Serial Cardiac Enzyme Data: A Clinical Application of Artificial Machine Intelligence", pages 134-141, see entire document.	1-3,5
X	CLINICAL CHEMISTRY, Volume 38, Number 1, issued 1992, M.L. Astion et al, "Application of Neural Networks to the Interpretation of Laboratory Data in Cancer Diagnosis", pages 34-38, see entire document.	1-5
X	CANCER LETTERS, Volume 77, issued 1994, P. Wilding et al, "Application of Backpropagation Neural Networks to Diagnosis of Breast and Ovarian Cancer", pages 145-153, see entire document.	1-5
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA, Volume 88, issued December 1991, G. Reibnegger et al, "Neural Networks as a Tool for Utilizing Laboratory Information: Comparison with Linear Discriminant Analysis and with Classification and Regression Trees", pages 11426-11430, see entire document.	1-3,5